Reducing height and lodging in canola (*Brassica napus* L.) using plant growth regulators

by

Lambertus Lochner Eksteen

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Supervisor: Prof André Agenbag

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Lambertus Lochner Eksteen

Abstract

In South Africa, canola (*Brassica napus* L.) is produced under short day conditions during winter months. These conditions, together with high fertiliser application levels required to maximize grain yields, often result in tall growing bulky crops which are prone to lodging. This will especially be true if canola production is expanded to irrigated areas. Plant growth regulators (PGRs) have successfully been used to reduce canola plant height and lodging under experimental conditions in Australia and are worldwide commercially used to reduce plant height and lodging in winter cereals such as wheat and barley. The primary objective of this study was to determine the effect of anti-lodging PGRs on the agronomic and quality characteristics of commercial canola cultivars under South African conditions.

This study was conducted under field conditions at three research farms, as well as controlled glasshouse conditions at Welgevallen Research Farm, situated in the Western Cape Province of South Africa. Foliar treatments consisted of a control (untreated) and four PGRs; CeCeCe® 750 (chlormequat chloride), Moddus® 250 EC (trinexapac-ethyl), Primo MAXX® (trinexapac-ethyl), and Kelpak®, applied either individually or in combination with wetting agent at budding stage (growth stage 3.1) of canola. Whilst glasshouse trials were conducted with spring canola cultivars “Hyola 555TT” and “43C80”, field trials were done with Hyola 555TT only. Monitoring and measuring various plant parameters during different growth stages of canola, the morphological and physiological impact of PGR-treatments on growth and development were determined.

Though Primo MAXX® tends to reduce plant height in all trials; reductions were only significant during one of the glasshouse trials. Fortunately, compared to the control, none of the PGRs significantly reduced the leaf area, number of flowers or number of pods plant^{-1} during this study, while Primo MAXX® and Moddus® 250 EC tend to increase the grain yield under field conditions. This study indicates that PGRs can possibly be used to improve lodging resistance and yield of canola. Identifying the most effective PGRs on specific cultivars, the results of the study will contribute to the knowledge of using PGRs in canola to reduce lodging and improve grain yields in South Africa.

Uittreksel

In Suid-Afrika word canola (*Brassica napus* L) gedurende die wintermaande onder kort-dagtoestande verbou. Hierdie verbouingstoestande, tesame met verhoogde toediening van bemesting vir maksimum opbrengs, lei dikwels tot welige, hooggroeiende plantegroei, wat 'n geneigdheid toon om om te val (lodge). Hierdie probleem vererger indien canola onder besproeiingstoestande gekweek sou word. Plantgroeireguleerders (PGRs) is reeds met sukses onder eksperimentele toestande in Australië gebruik om die planthoogte en dus omval (lodging) van canola te beperk. Plantgroeireguleerders word reeds kommersieel gebruik om planthoogte en omval van wintergraangewasse, soos koring en gars te verminder. Die hoofdoel van hierdie studie was om die effek van omval-teenwerkende PGRs op die agronomiese en kwaliteitseienskappe van kommersiële canola-kultivars onder Suid-Afrikaanse groeitoestande te bepaal.

Hierdie studie is uitgevoer d.m.v. veldproewe op drie navorsingsplase, sowel as gekontrolleerde glashuisproewe te Welgevallen Navorsingsplaas, geleë in die Wes-Kaapprovinsie van Suid-Afrika. Blaartoedienings het bestaan uit 'n kontrole (onbehandeld) en vier PGRs: CeCeCe® 750 (chlormequat chloride), Moddus® 250 EC (trinexapac-ethyl), Primo MAXX® (trinexapac-ethyl) en Kelpak®, wat afsonderlik of in kombinasie met benattingsmiddels toegedien is tydens die blomknopverskyningstadium (groeistadium 3.1) van canola. Die glashuisproewe is uitgevoer met lente-canolakultivars, nl. "Hyola 555TT" en "43C80", terwyl veldproewe slegs uitgevoer is met Hyola 555TT. Verskeie plantparameters is gemonitor en gemeet gedurende die verskillende groeistadia van canola, waartydens en die morfologiese en fisiologiese impak van PGR-toedienings op die groei- en ontwikkeling van canola bepaal is.

Alhoewel Primo MAXX® neig om die planthoogte in al die proewe te verkort, het dit planthoogte slegs beduidend verkort in een van die glashuisproewe. Geen van die PGRs het in vergelyking met die kontrole, die blaar-oppervlakte, aantal blomme of aantal peule plant^{-1} beduidend verminder gedurende die studie. Primo MAXX® en Moddus® 250 EC het inteendeel daartoe geneig om die saadopbrengs onder veldtoestande te verhoog. Hierdie studie toon dus dat PGRs moontlik gebruik kan word om omval te verminder en die opbrengs van canola te verhoog. Deur die mees doeltreffendste PGRs op spesifieke kultivars te identifiseer, kan die resultate van hierdie studie bydra tot die kennis van die gebruik van PGRs op canola, om omval te voorkom en saadopbrengs in Suid-Afrika te verhoog.

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Chapter 1
Introduction

Canola, *Brassica napus* L. (Brassicaceae), was introduced as a commercial crop in South Africa in 1994 when about 5 000 ha was planted (Mosiane et al. 2003). Since then canola has become, as in the rest of the world, an important temperate oil crop of economic value, producing a yield of 112 014 tons in South Africa on 72 165 ha during 2013 (Ijaz and Honermeier 2012; Crop estimates 2014).

In South Africa, canola (*Brassica napus* L.), is produced under short day conditions during winter months (Mosiane et al. 2003). These conditions, together with high fertiliser application levels required to maximize grain yields, often result in tall growing bulky crops which are prone to lodging. This will especially be true if canola production is expanded to irrigated areas. Plant growth regulators (PGRs) have successfully been used to reduce canola plant height and lodging under experimental conditions in Australia (Armstrong and Nicol 1991; Ramburan and Greenfield 2007a) and are worldwide commercially used to reduce plant height and lodging in winter cereals such as wheat and barley.

Lodging is a significant risk in intensively grown canola, resulting in stem bending and downfall of bulky crops with large biomass and seed yields, especially during pod development, heavy precipitation and strong winds (Armstrong and Nicol 1991; Gebre et al. 2010). Lodging reduces the supply of assimilates, photosynthesis, harvesting efficiency, grain-filling, -quality and -yield, while enhancing disease severity and pod shattering in canola (Armstrong and Nicol 1991; Ramburan and Greenfield 2007b; Wiersma et al. 2011; Gebre et al. 2012; Ijaz and Honermeier 2012).

Improving resistance with regard to lodging is one of the most important characteristics in high fertility and irrigated practices (Syme 1969). According to Armstrong and Nicol (1991) and Li et al. (2011) shorter plants require smaller quantities of water and nutrition and are considerably more resistant towards lodging than taller plants. In view of the fact that a reduction in main shoot growth may reduce the competition for assimilates and light, the availability of unused growth resources to growing organs of competing plant parts may be enhanced, thus potentially producing a more compact and even pod canopy (Armstrong and Nicol

1991; Rajala et al. 2002). In addition shorter, erect plants will most likely improve harvesting efficiency while producing greater yields, especially under extreme drought conditions (Armstrong and Nicol 1991; Li et al. 2011). However, in few cases shorter plants produce an equal grain yield (Armstrong and Nicol 1991). Thus the question derives; how can canola height be shortened to improve resistance towards lodging?

Recently, semi-dwarf canola cultivars have restricted losses caused by lodging. Nevertheless, the problem has not yet been eliminated since even late seasonal semi-dwarf cultivars endure lodging under lodging-promoting environmental conditions and intensive management inputs, as in the case of wheat (Ramburan and Greenfield 2007b).

According to Matysiak and Kaczmarek (2013), research confirmed a significant reduction in plant height when applying PGRs to canola growing in high sowing densities. However, high sowing densities may result in reduced sprouting, while enhancing sowing costs, competition for assimilates and the susceptibility towards fungal diseases (Matysiak and Kaczmarek 2013).

According to Basra (2000): “Plant growth regulators are organic compounds other than nutrients (supplying either energy or mineral elements) that, in small amounts, promote, inhibit, or otherwise modify any physiological process in plants”. Thus far PGRs have successfully reduced plant height and lodging in intensively grown cereals, while maintaining grain yield (Rajala et al. 2002; Matysiak 2006; Gebre et al. 2010; Wiersma et al. 2011). In Australia, Armstrong and Nicol (1991) accordingly reported similar results on canola, along with several other benefits including more uniform ripening, enhanced weeds suppression and a reduction in pod shattering. However, in South Africa the use of anti-lodging PGRs is restricted due to the shortage of scientific data regarding their application on commercial canola cultivars.

Due to problems such as uneven ripening, harvesting problems and possible yield losses associated with lodging which may hamper canola production in South Africa, a study was conducted to investigate the possibility of using PGRs as anti-lodging agents in canola production.

The main objectives of this study

To determine the potential of PGRs as anti-lodging agents in canola by measuring their effects on the agronomic and quality characteristics of two different canola cultivars under glasshouse conditions.

To determine the effect of anti-lodging PGRs agents on the agronomic and quality characteristics of canola under field conditions at different localities in the Western Cape.

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Chapter 2
Literature Review

2.1. Introduction

Since the 1930s, cultivated crops have been modified to obtain specific advantages, using plant growth regulators (PGRs) (Rademacher and Brahm 2012). According to Basra (2000): “Plant growth regulators are organic compounds other than nutrients (supplying either energy or mineral elements) that, in small amounts, promote, inhibit, or otherwise modify any physiological process in plants”. However, for practical purpose the term PGR not only includes organic compounds (phytohormones) but synthetic compounds (chemical analogs). Both compounds regulate plant growth and development by means of alternating the balance of hormones in target plants, thus manipulating both physiological and morphological reactions (Nickell 1982; Basra 2000).

The following phytohormones and synthetic plant growth regulators may be of great importance in the reduction of both plant height and lodging in canola, *Brassica napus* L. (Brassicaceae).

2.2. Auxins

During the 1920s Frits Went discovered an unknown compound, causing curvature of coleoptiles toward light (phototropism), later identified as auxin. In Greek the word “auxin” bares the meaning to increase (Salisbury and Ross 1991). It was not until 1946 that pure indole-3-acetic acid (IAA) was isolated from the endosperm of immature corn grains, today known as primary auxin present in plants (Vivanco and Flores 2000). Contributing to plant growth as its main hormonal function, auxin play a significant role in several physiological reactions including cell division and elongation, phototropism, gravitropism, apical dormancy, activation of cambial growth, promotion of adventitious root formation and the formation of abscission layer on fruit and leaves (Salisbury and Ross 1991; Vivanco and Flores 2000; Hartmann et al. 2002; Rademacher and Brahm 2012). Since 1946 three other isolates bearing similar structures and responses have been identified: phenylacetic acid (PAA), indole-3-butyric acid (IBA) and 4-chloro-IAA (Salisbury and Ross 1991; Vivanco and Flores 2000).

Manufactured in developing seeds, young leaves and leaf primordia, auxin (IAA) can be biosynthesized via two mechanisms (Salisbury and Ross 1991; Hartmann et al. 2002; Bore and Ng´etich 2007). Even though one is more common than the other, both mechanisms involve the removal of the terminal carboxyl group and amino-acid group from tryptophan's side chain. The most common pathway starts off by donating the amino group to α-keto acid, which will thereafter be converted into indolepyruvic acid via a transamination reaction. This is followed by a decarboxylase reaction to form indoleacetaldehyde which will eventually be oxidized to IAA (Salisbury and Ross 1991). After that biosynthesized auxins is transported via a polar gradient through vascular bundles (parenchyma cells) towards the roots (Salisbury and Ross 1991; Hartmann et al. 2002; Bore and Ng´etich 2007), triggering the elongation of primary root, lateral root formation, and gravity response (Gavelienė et al. 2007).

Two types of auxins occur within plants, free and bound auxins. Unlike bound auxins, free auxin can regulate physiological processes immediately after rapidly diffusing out of tissue (Salisbury and Ross 1991; Vivanco and Flores 2000). Before bound auxin can be conjugated to available amino acid, carbohydrates or peptides, it is required to be subjected to autolysis, enzymolysis or hydrolysis. Bound auxin usually serves as storage, reserve (glucosides) and detoxification forms of auxin (Salisbury and Ross 1991). In contrast to the formation of auxins, two degradative types of removal processes have been identified. The first type involves the oxidization of IAA by oxygen (O_2), during which the carboxyl group will be lost as carbon dioxide (CO_2). During the second type the heterocyclic ring's carbon 2 will be oxidized to oxindole-3-acetic acid (Salisbury and Ross 1991).

For auxin to be used as a PGR, it is of utmost importance to be absorbed by leaves mature enough to export sugars, as they are able to transport exogenous auxins from the surface through the sieve tubes (Salisbury and Ross 1991).

2.2.1. Dehiscence zone of canola pods

As soon as canola pods reach maturity, they are susceptible to dehiscence also known as "pod shattering". During pod shattering the dehiscence zone, a discrete layer of cells sealing the separation layer, suffers the loss of cellular cohesion, thus bringing about shattering of pods and the release of grain. In the course of adverse

weather conditions, the potential canola yield can be reduced up to 50% due to pod shattering (Chauvaux et al. 1997).

Pod shattering is in distinct resemblance to abscission of canola fruit, flowers and leaves (Chauvaux et al. 1997). According to Chauvaux et al. (1997) abscission is inhibited by auxin whereas promoted by ethylene. During the development of the separation layer, cellulases (β-1,4-glucanases) activity increase substantially in reaction to ethylene. Increased β-1,4-glucanase activity is linked with the reduction of auxin content. Nevertheless treating canola pods with synthetic auxins such as 2-methyl-4-chlorophenoxyacetic acid (4-CPA), the activity of β-1,4-glucanase along with maturation and senescence could possibly be delayed with approximately 10 days (Chauvaux et al. 1997).

2.3. Cytokinins

During 1913 (Austria), Gottlieb Haberlandt discovered that cell division can be stimulated by an unknown compound present in vascular tissues of various plants. Although discovered by Haberlandt, a professor named Skoog (Wisconsin University) identified the unknown compound as cytokines during his studies, developing methods for growing plant tissues aseptically in culture (Salisbury and Ross 1991; Hartmann et al. 2002). Promoting cell division as their main hormonal function, cytokinins (N^6-substituted adenine derivatives) appear to be one of the most vital plant hormones to exist (Vivanco and Flores 2000; Hartmann et al. 2002; Bore and Ng´etich 2007; Rademacher and Brahm 2012).

Synthesized in the root cap and meristematic cells surrounding the quiescent center of the root tip, cytokinin biosynthesis involves the initial steps of the mevalonic acid pathway up until the isopentenyl pyrophosphates steps. The cytokinin biosynthesis pathway starts off by converting isopentenyl pyrophosphate and adenosine monophosphate (AMP) into isopentenyl AMP. Afterwards isopentenyl AMP is transformed into isopentenyladenosine, followed by a series of metabolic steps which eventually produce cytokinins (Salisbury and Ross 1991; Vivanco and Flores 2000). Transported via the xylem to the rest of the plant parts, cytokinins mainly accumulate in young organs such as leaves, fruit, seeds and shoots (Salisbury and Ross 1991; Vivanco and Flores 2000; Bore and Ng´etich 2007). In the shoot, cytokinins play a significant role regulating photosynthesis, timing and growth of

senescence (Vivanco and Flores 2000). Moreover cytokinins can also be retransported from mature leaves to younger organs via the phloem (Salisbury and Ross 1991).

As in the case of auxins there are two types of cytokinins occurring within plants, bound and free cytokinins. Bound cytokinins consist the potential to be produced in different ways, such as alanine or glucosides conjugates. Alanine conjugates known as irreversible formed products, play a substantial part in the detoxification mechanisms of plants. Glucoside conjugates function as a storage form or it may facilitate the transport of certain cytokinins. On the other hand, free cytokinins are represented by zeatin and isopentenyladenine, some of the most physiologically active and naturally-occurring cytokinins in plants (Salisbury and Ross 1991; Vivanco and Flores 2000; Rademacher and Brahm 2012). Nevertheless, zeatin and isopentenyladenine are not of practical relevance. Comprising over cytokinin-like activity, diphenylurea derivatives (e.g. chlorflorfenuron and thidiazuron) and adenine-type compounds (e.g. benzyladenine and kinetin) are of commercial use (Hartmann et al. 2002; Rademacher and Brahm 2012). According to Salisbury and Ross (1991), exogenous applied cytokinins can only promote plant growth (incl. stem elongation) significantly if tissues (coleoptiles) are still young and cell division still occurs.

Cytokinins can also be inactivated by certain reactions which occur during the configuration of N-conjugates with alanine or glucose, and the oxidative cleavage of N^6 side chain of the cytokinin substrate by cytokinin oxidase (Vivanco and Flores 2000).

Kelpak®

Controlling the relationship between different plant hormones, plant growth and development can be manipulated according to mankind's liking. For example, whilst shoot formation is favored by a high cytokinin: auxin ratio, rooting is favored by the reverse (Sachs 2005; Bore and Ng´etich 2007).

Containing a wide variety of plant growth-stimulating compounds, commercial seaweed concentrates have been extensively used on agricultural crops affecting their cellular metabolism, thereby enhancing plant growth and yield (Khan et al. 2009). During the 1940s the first known liquid seaweed extract used for agricultural purposes was produced and marketed as Maxicrop®. Since then numerous other

seaweed extracts have been developed and used as plant bio-stimulants (Stirk et al. 2014). Kelp Products (Pty) Ltd. have been produced in South Africa (Simon's Town) since 1979 (Stirk et al. 2014). The liquid seaweed extract Kelpak® is produced from the kelp *Ecklonia maxima* (Osbeck) Papenfuss, by means of a "cold cell burst method" (Nelson and Van Staden 1984; Ferreira and Lourens 2002; Robertson-Andersson et al. 2006; Bore and Ng´etich 2007; Stirk et al. 2014). During the extraction method pressure is rapidly changed, thus rupturing cells and releasing cellular contents. Unlike most seaweed extracts Kelpak® is produced without using acidic or alkaline solutions at high temperatures which could potentially destroy cellular compounds (Robertson-Andersson et al. 2006; Papenfus et al. 2012; Stirk et al. 2014).

Commercially Kelpak® is registered as a natural water soluble concentrate plant growth regulator (bio-stimulator) used on a broad variety of crops, including canola (Ferreira and Lourens 2002; Robertson-Andersson et al. 2006; see Kelpak® label). According to Stirk et al. (2014) Kelpak® includes various types of auxins, cytokinins and polyamines. Containing natural regulators high in auxins (11.0 mg L^{-1}) and low in cytokinins (0.031 mg L^{-1}), Kelpak® has been reported to improve the root growth and development of various crops including nutrient-stressed okra seedlings (Papenfus et al. 2012), cabbage, marigold, mung bean, tomato and wheat (Khan et al. 2009) as a result of the well-known effect caused by the root growth promoting hormone auxins. Improving lateral root formation and volume of the total root system, the efficiency of water and nutrient uptake are enhanced, hence promoting the overall plant growth (incl. plant height, leaf area, chlorophyll content, lower node diameter, strengthening of the stem and dry mass) as well as subsequent yield quantity and quality of various agricultural crops (Nelson and Van Staden 1984; Ferreira and Lourens 2002; Robertson-Andersson et al. 2006; Bore and Ng´etich 2007; Khan et al. 2009; Papenfus et al. 2012; Papenfus et al. 2013; Stirk et al. 2014). Khan et al. (2009) further state that cytokinin is associated with nutrient mobilization in both vegetative and reproductive organs. Elevating cytokinin levels and availability, seaweed extracts may possibly be responsible for increased cytokinin mobilization from roots to developing fruit, or an improved quantity or synthesis of endogenous fruit cytokinins (Khan et al. 2009). Improved yield quality and quantity due to Kelpak® applications were previously reported in barley, beans (24% yield increase), peppers

and wheat grown under potassium stress (Khan et al. 2009) and under growth chamber conditions (Nelson and van Staden 1984). Additional benefits detected in several plant growth bioassays include the treated plant's ability to withstand adverse biotic and abiotic stresses (incl. nematode infestation) (Nelson and Van Staden 1984; Bore and Ng´etich 2007; Khan et al. 2009).

According to Khan et al. (2009) seaweed extracts not only stimulate direct plant growth, but also improves biological, chemical and physical soil properties. Alginates known as a polyuronides occur opulently in the cell wall of brown seaweeds (incl. *Ecklonia maxima*) (Robertson-Andersson et al. 2006; Khan et al. 2009). Forming high-molecular-weight complexes, alginates affect soil properties combining salt from their free acid form (alginic acid) together with metallic ions present in soil. By way of absorbing and retraining moisture these complexes promote moisture-holding capacity, crumb structure, soil aeration, soil pore capillary-activity as well as beneficial soil microbial activity (Khan et al. 2009).

According to the directions for use on wheat, barley, oats and canola, a 2.0 L ha^{-1} Kelpak® dosage needs to be applied as a ground- (up to 500 L water ha^{-1}; in combination with a wetter/sticker) or areal-application (30 L water ha^{-1}) between the 3 to 5 leaf stage (wheat growth stage 7 according to Centre of Small Grain reference list) (see Kelpak® label). In general liquid seaweed extract are recommended for foliar application, given that cytokinins are absorbed through the leaf surfaces (Ferreira and Lourens 2002). According to Ferreira and Lourens (2002) Kelpak® can be exogenously applied on its own or in conjunction with herbicides at numerous early growth stages in canola. Usually Kelpak is applied in combination with herbicide mixtures and a wetter/sticker (see Kelpak® label), for cost saving and improved absorbed purposes.

2.4. Gibberellins

During 1935 active crystalline material was isolated from *Gibberella fujikoroi* (foolish seedling disease on rice), a disease causing rice plants to significantly increase in plant height and eventually fall over. After applying this substance, later called gibberellin A, to the roots of rice seedlings, plant growth was miraculously stimulated (Vivanco and Flores 2000). Today only a few of the 100 forms of gibberellins (GAs) identified are known to be biological active, whereas the rest are to be catabolites or

precursors (Rademacher 2000; Vivanco and Flores 2000). As diterpenoids consisting out of 19 or 20 carbon atoms (Rademacher 2000), GAs can only be activated in the presence of a 3β-hydroxyle group (Vivanco and Flores 2000).

The gibberellin biosynthesis pathway can be separated into the following main phases: (1) the conversion of mevalonic acid to the C20 compound geranylgeranyl diphosphate (GGPP); (2) the cyclization of GGPP as donor of gibberellin carbon atoms, into copalyl diphosphate (CPP) and then *ent*-kaurene, (3) the oxidization of *ent*-kaurene via *ent*-kaurenal and *ent*-kaurenol into ent-kaurenoic acid which will thereafter be transformed to GA_{12}-aldehyde, (4) the conversion of GA_{12}-aldehyde (20-carbon molecule) into different GAs (Salisbury and Ross 1991; Rajala and Peltonen-Sainio 2000). Nevertheless, unique pathways are being used by different plant species to produce distinct GAs (Rademacher 2000; Vivanco and Flores 2000).

Within the plant, GAs is subsequently transported via non-polar pathways (xylem and phloem) from the main site of gibberellin biosynthesis (young leafs) to the sink. Take the case of *Phaseolus coccinensis* for example; after GA_{19} have been manufacture inside the shoots, it is translocated into the roots where it will be converted to GA_1. Thereafter GA_1 will be retranslocated back into the shoots (Vivanco and Flores 2000). According to Rademacher (2000) and Hartmann et al. (2002) the prime hormonal functions of GAs include the initiation of bolting in long-day plants, the initiation of hydrolytic enzymes in germinating seeds, hence the high concentrations present, as well as the stimulation of fruit set, development and longitudinal growth of plants. Gibberellins stimulate stem elongation mainly through enhancing cell elongation rather than cell division (Rajala and Peltonen-Sainio 2000; Vivanco and Flores 2000). Commercially GAs is used to promote the distance between grape branches and the size of seedless-grape berries, utilizing the unique ability to promote extensive growth of intact plants (Salisbury and Ross 1991). Therefore GAs is known to coordinate the total plant growth (Vivanco and Flores 2000).

The age and certain environmental properties determine the time at which a plant forms flowers. In certain species GAs can overcome the inductive cold period (vernalization) required to flower or flower sooner, whereas in other species GAs can substitute for long-day requirement, showing an interaction with light (Salisbury and

Ross 1991). According to Salisbury and Ross (1991), some gibberellins are much more effective in enhancing flowering than others.

2.4.1. Anti-gibberellins

To overcome lodging, one of the main goals in breeding canola and cereal is shortening of the stem length (Rademacher 2000; Rajala and Peltonen-Sainio 2000). This can primarily be achieved using PGRs containing anti-gibberellins, also known as gibberellin biosynthesis inhibitors (Nelson and Van Staden 1984; Rajala and Peltonen-Sainio 2000). Blocking the gibberellin biosynthesis pathway at various stages, anti-gibberellins affect the balance of plant hormones. As a result the rate of both cell elongation and division is reduced; hence inhibiting internode elongation and reducing both plant height and risk of lodging (Nelson and Van Staden 1984; Rajala and Peltonen-Sainio 2000; Ramburan and Greenfield 2007a; Gebre et al. 2010; Spitzer et al. 2011; Rademacher and Brahm 2012).

Four groups of anti-gibberellins are known which interfere with gibberellin biosynthesis at different stages: onium-type compounds, compounds with a nitrogen-containing heterocycle, structural mimics of 2-oxoglutaric acid, and 16,17-dihydro-GAs (Rademacher 2000).

2.4.1.1. Onium-type compounds

Onium-type compounds, compounds that possess a positively charged sulphonium, phosphonium or ammonium group block the GA biosynthesis prior to the formation of *ent*-kaurene. Mepiquat chloride and chlormequat chloride are two of the most well-known compounds (anti-gibberellins) representing this group (Rademacher 2000). According to Rademacher (2000) mepiquat chloride and chlormequat chloride inhibit both CPP-synthase and *ent*-kaurene synthase, via mimicking cationic intermediates and binding firmly to the enzymes. As a result the conversion of GGPP into CPP and CPP into *ent*-kaurene is blocked during the second main phase of gibberellin biosynthesis (see above) (Rademacher 2000). Commercially onium-type compounds are most commonly used as stem-shortening PGRs for the control of lodging in modern high input cereal management (Armstrong and Nicol 1991; Rajala et al. 2002; Ramburan and Greenfield 2007b; Rademacher and Brahm 2012). Trade name: CeCeCe® 750 and Pix (BASF®).

CeCeCe® 750

Since its introduction in 1965, anti-gibberellin compound chlormequat chloride became the new standard anti-lodging PGR, most often used for the control of lodging in modern high input cereal management (Rajala et al. 2002; Ramburan and Greenfield 2007b; Rademacher and Brahm 2012). Containing 750 g L^{-1} chlormequat chloride active ingredient, water soluble solution PGR CeCeCe® 750 is registered (BASF® South Africa) for the control of wind damage and lodging in wheat and excessive shoot growth in pears (see CeCeCe® 750 label).

Containing onium-type compounds, chlormequant chloride blocks cyclases involved in early stages of gibberellic acid biosynthesis, thus inhibiting the activity of *ent*-kaurene synthesis (Gebre et al. 2010; Žiauka and Kuusienė 2010; Gebre et al. 2012; Rademacher and Brahm 2012). As a result the rate of both cell elongation and division is reduced, hence inhibiting internode elongation and reducing both plant height and risk of lodging (Ramburan and Greenfield 2007a; Gebre et al. 2010; Spitzer et al. 2011). Numerous previous studies done on chlormequat chloride (CeCeCe® 750) alone or in PGR combinations support a reduction in internode elongation (Armstrong and Nicol 1991; Giridhar and Giri 1997; Sanvicente et al. 1999; Gans et al. 2000; Rajala and Peltonen-Sainio 2001; Aamlid et al. 2007; Haque et al. 2007; Zhang et al. 2009; Spitzer et al. 2011; Gebre et al. 2012; Matysiak and Kaczmarek 2013).

According to Rajala et al. (2002) the reduction in main shoot growth induced by chlormequat chloride may result in the availability of unused growth resources to growing organs of competing plant parts (e.g. tillers and roots). Improving root growth and development (Cycoń et al. 2012), chlormequant chloride treated plants may extract water more efficiently from deeper soil levels; hence better utilisation of available resources (Rajala and Peltonen-Sainio 2001). As a result overall plant growth is enhanced including root size, leaf area $plant^{-1}$ (Cycoń et al. 2012), culm diameter; dry mass (Sanvicente et al. 1999; Gebre et al. 2012) as well as subsequent grain yields (Giridhar and Giri 1997; Rajala and Peltonen-Sainio 2001; see CeCeCe® 750 label). Giridhar and Giri (1997) furthermore report a potential improvement in both protein and oil yields after spraying groundnut plants with “CCC” (chlormequat chloride applied at 0.5 mL L^{-1} water). However, according to

previous studies chlormequat chloride had the tendency to delay onset of flowering and pod development (Spitzer et al. 2011).

According to the directions for use on wheat, stem-shortening PGR CeCeCe® 750 needs to be operated as a foliar spray at the beginning of stem elongation (5 to 7 leaf stage), either as a ground- (300 to 400 L water ha^{-1}) or aerial-application (30 L water ha^{-1}) (see CeCeCe® 750 label).

PIX®

Plant growth regulator PIX® (BASF®) is a foliar-applied water soluble solution registered for the reduction of vegetative growth in cotton (Rademacher and Brahm 2012, see PIX® label). Also used in onions, garlic and grapevines (Rademacher and Brahm 2012), active ingredient mepiquat chloride (50 g L^{-1}) may result in one or more of the following effects: darker green leaves, more open canopy, height reduction, shorter limbs, improved boll retention and earlier maturity. As a result the percentage mature cotton seeds along with yield potential may be enhanced while facilitating earlier harvesting simultaneously (see PIX® label). According to previous studies done on the reduction of plant height and lodging in canola (Armstrong and Nicol 1991), “Pix” (5% w/v a.i. mepiquat chloride at 1.0 L ha^{-1}) did not have significant effects on the plants characters.

According to the directions for use, PIX® can be applied at three different occasions: first application applied during the early flower bud growth stage; the follow-up treatment applied with intervals of approximately two weeks depending on the growth rate; and the final treatment applied at the end of the effective flowering period. Furthermore PIX® may be applied as a ground- (min of 200 L water ha^{-1}) or aerial-application (40 L water ha^{-1}) (see PIX® label).

2.4.1.2. Compounds with a nitrogen-containing heterocycle

The third main phase of the GA biosynthesis pathway may also be inhibited via compounds consisting of a nitrogen-containing heterocycle, such as the closely related growth retardants, uniconazole, uniconazole-P and paclobutrazol. Commercially these growth retardants have been found useful controlling vegetative growth of certain fruit trees (incl. avocados and mangos), controlling lodging in rice, and enhancing more compact production of ornamentals (Rademacher 2000;

Rademacher and Brahm 2012). Structurally the heterocyclic rings of all these compounds have one distinct feature in common, a lone pair of electrons on the sp^2-hybridized nitrogen (Rademacher 2000). According to Rademacher (2000) the lone pair of electrons acts by displacing oxygen from its binding site, thereby blocking the active site of monooxygenases which is the enzyme responsible for *ent*-kaurene oxidation. Finally the conversion of *ent*-kaurene to *ent*-kaurenoic acid is inhibited. Nevertheless, steps involving monooxygenases after the production of *ent*-kaurenoic acid seemed to stay unaffected (Rademacher 2000). Trade name: ®Sunny* 50 SC (Philagro®) and Cultar® (Syngenta®).

®Sunny* 50 SC

Plant growth regulator ®Sunny* 50 SC (Uniconazole 50 g L^{-1}) is a registered trademark of Aquamarine (London), limited and sold by Philagro® in South Africa. Registered on avocados in South Africa, the anti-gibberellin ®Sunny* 50 SC is used to control vegetative growth as well as improvement of fruit shape. According to directions for use, ®Sunny* 50 SC should be applied in combination with foliar feed "UP 50" or a registered anionic wetting agent. The PGR should be applied at flowering (1.0 to 2.0 L ®Sunny* 50 SC and 2.0 L UP 50 or 0.05 L anionic wetting agent 100 L^{-1} spray mixture) or after mid-summer pruning (500 mL ®Sunny* 50 SC and 50 mL ®Sanawett* 90 to 940 SL 100 L^{-1} spray mixture), given vegetative growth is between 10 to 15 cm long (see ®Sunny* 50 SC label).

Cultar®

Cultar® is a systemic PGR registered on apples and pears and trademark of the chemical company Syngenta®. Containing active ingredient paclobutrazol (250 g L^{-1}), Cultar® acts by reducing the internodal length of newly developed shoots along with earlier formation of terminal buds. As a result fruit bud production, quality and harvest yield may be improved. However due to residual soil activity Cultar® may affect growth of the following crops, resulting in yield loss (see Cultar® label).

2.4.1.3. Structural mimics of 2-oxoglutaric acid

Acylcyclohexanediones such as trinexapac-ethyl and prohexadione-calcium represent part of the inhibitory-group, "structural mimics of 2-oxoglutaric acid", responsible for blocking the late steps of GA biosynthesis. Due to the high degree of

structural similarities between acylcyclohexanediones and the co-substrate of dioxygenases involved 2-oxoglutaric, the activity of dioxygenases is blocked during the fourth main phases of GA biosynthesis (see above). Mainly targeting hydroxylations at position 3ß and 2ß, the free acid form of acylcyclohexanediones inhibit most GA biosynthesis steps after GA_{12}-aldehyde, including the conversion of GA_{20} into GA_1 and GA_1 into GA_8 (Rademacher 2000; Rademacher and Brahm 2012). Resulting in reduced shoot growth in higher plants, trinexapac-ethyl and prohexadione-calcium are used commercially stabilizing stems and controlling lodging in rice, cereals and canola, and reducing vegetative growth in turf grasses and fruit trees (esp. pome fruit) (Rademacher 2000; Rademacher and Brahm 2012). Trade name: Regalis® (BASF®), Primo MAXX® and Moddus® 250 EC (Syngenta®).

Regalis®

Comprising active ingredient prohexadione-calcium (100 g kg^{-1}), Regalis® (BASF®) is a water dispersible granular PGR used to reduce excessive shoot growth in fruit trees (e.g. apples) and peanuts (Rademacher and Brahm 2012; see Regalis® label). Benefits may include less pruning, enhanced fruit set as well as more efficient harvesting (Rademacher and Brahm 2012). Since foliar absorbed, the PGR should be applied to active growing trees (according to tree size) at a minimum rate of at least 1.25 kg ha^{-1} in combination with “Dash HC” (70 g Regalis® 100 L^{-1} water and 60 mL Dash HC 100 L^{-1} water). The first application needs to be sprayed at growth stage 31 (100% petal drop), during which the terminal shoot are approximately 5 to 7 cm long and 3 to 5 leaves are fully developed $shoot^{-1}$. The second application is required four weeks later (see Regalis® label).

Primo MAXX® and Moddus® 250 EC

Primo MAXX® and Moddus® 250 EC are both anti-gibberellin PGRs and registered trademarks of Syngenta®. Turf growth regulator Primo MAXX® is registered for the reduction of stem and leaf growth of grass species, whereas Moddus® 250 EC, not yet registered in South Africa, is used for the reduction in plant height, lodging prevention and yield protection on the following seed crops: spring and winter -oats, -barley, -wheat, durum wheat, triticale, grasslands and rye. Both PGRs contain active ingredient trinexapac-ethyl, however differing in dosages. According to their labels Primo MAXX® is a micro emulsion concentrate containing 120 g L^{-1} trinexapac-ethyl,

while Moddus® 250 EC is an emulsifiable concentrate containing 250 g L^{-1} trinexapac-ethyl (see Primo MAXX® and Moddus® 250 EC labels).

Interfering with the late stages of gibberellic acid biosynthesis, trinexapac-ethyl structurally mimics 2-oxoglutaric acid, the co-substrate of dioxygenases engaged. As a result the conversion of GA_{20} to GA_1 (active gibberellins) is inhibited, bringing about an increased concentration of GA_{20} and total non-structural carbohydrates while decreasing cell elongation (Lickfeldt et al. 2001; McCann and Huang 2007; Kreuser and Soldat 2011; Wiersma et al. 2011).

Blocking the gibberellic acid metabolism, plant growth and development may furthermore be alternated, reducing plant height (Matysiak 2006; Li et al. 2011; Wiersma et al. 2011; Ijaz and Honermeier 2012) and dry matter production, while at the same time enhancing chlorophyll concentration, mesophyll cell density, leaf area, tiller density, root growth, stem diameter and lignin accumulation in stem cells, hence improving stem strength (Matysiak 2006; Espindula et al. 2009; Rolston et al. 2010; Kreuser and Soldat 2011; Wiersma et al. 2011). Nevertheless reports of trinexapac-ethyl effects on plant parameters e.g. root growth, have varied from improvement (Ijaz and Honermeier 2012), to no response (Rajala et al. 2002; McCann and Huang 2007). Producing shorter erect plants, trinexapac-ethyl may be used as a stem-shortening PGR restraining lodging and yield losses (McCann and Huang 2007; Rolston et al. 2010; Li et al. 2011; Wiersma et al. 2011; Ijaz and Honermeier 2012). Although trinexapac-ethyl applications may result in crop injury (chlorosis) for a short period (Wiersma et al. 2011), additional advantages may include enhanced drought tolerance (McCann and Huang 2007), increased grain yield (Matysiak 2006; Espindula et al. 2009), flexibility in timing of application and long-lasting effects (Wiersma et al. 2011). Furthermore Ijaz and Honermeier (2012) pointed out a potential increase in seed size, linoleic- (20.0%) and linolenic-acid (10.4%) concentrations of canola (*B. napus* L.) treated with “Moddus” 222.0 g L^{-1} trinexapac applied at 0.5 L ha^{-1}.

Turf management tool, Primo MAXX® is mainly used on golf courses, sport fields, commercial and residential lawns, cemeteries and other similar areas. According to the label Primo MAXX® needs to be applied in 100 to 500 L ha^{-1} water, using a flat fan nozzle. Using the recommended rates on turf, it ensures about 20 to 50% reductions in clippings over a period of 2 to 6 weeks. Nevertheless application may

be repeated after turf growth is resumed or more suppression is needed. Absorbed by leaves, germination and seedling growth is not affected by Primo MAXX® (see Primo MAXX® label). The application of Moddus® 250 EC on the other hand requires a minimum spray volume of 200 L ha^{-1} of water with a spray pressure of 2 to 3 bar. Depending on the crop habit and growth stage, an increase in water volume will be followed with an increased penetration of Moddus® 250 EC. The time of application for the above mentioned seed crops, varies from the leaf sheath erect stage to the growth stage before flag leaves are fully extended (see Moddus® 250 EC label).

2.4.1.4. 16,17-Dihydro-GAs

The fourth group of anti-gibberellins, most recently discovered are known as 16,17-dihydro-GAs. Similar to acylcyclohexanediones, 16,17-dihydro-GAs inhibits the activity of dioxygenases engaged in the fourth main phase of GA biosynthesis (e.g. 3ß hydroxylation). However, in contrast to acylcyclohexanediones, 16,17-dihydro-GAs simply tends to reduce the shoot elongation in specific plant species including *Lolium temulentum* and certain grasses (Rademacher 2000).

2.5. Conclusion

During 1991 the article “Reducing height and lodging in rapeseed with growth regulators”, was published in the *Australian Journal of Experimental Agriculture*. According to authors, Armstrong and Nicol (1991), anti-gibberellins were most efficient, producing shorter plants which ripened more uniformly, reduced pod shattering, overcame lodging, and even suppressed some weeds. More importantly, grain yields were maintained using PGRs. However, commercially, available information regarding possibilities for using anti-lodging PGRs on canola is restricted due to the shortage of scientific research conducted under South African conditions. For this reason a study was conducted to investigate the possibility of using PGRs as anti-lodging agents in canola production. By identifying the most effective PGRs, they can be used in canola management systems to possibly assist in lodging control in South Africa.

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Chapter 3

Influence of anti-lodging plant growth regulators on growth and yield of glasshouse grown canola (*Brassica napus* L.) in sandy soil

3.1. Introduction

Introduced in South Africa during 1994 (Mosiane et al. 2003), canola, *Brassica napus* L. (Brassicaceae), has rapidly grown into a leading temperate oil crop, producing a yield of 112 041 tons on a total of 72 165 ha during 2013 (Crop estimates 2014). Although well adapted to South Africa's marginal areas of temperate regions (Mosiane et al. 2003), tall cultivars along with high fertility and irrigation practices can produce bulky crops prone to lodging (Armstrong and Nicol 1991; Ramburan and Greenfield 2007a). Lodging generally affects productivity by decreasing the supply of assimilates, sprouting, grain-filling, -quality and -yield, while increasing disease severity and development of harvesting problems (Armstrong and Nicol 1991; Ramburan and Greenfield 2007b; Gebre et al. 2012).

Thus far plant growth regulators (PGRs) have successfully reduced plant height and lodging in intensively grown cereals, while maintaining grain yield (Rajala et al. 2002; Matysiak 2006; Gebre et al. 2010; Wiersma et al. 2011). In Australia, similar results have been reported on canola (Armstrong and Nicol 1991); however, in South Africa the use of anti-lodging PGRs are restricted due to the shortage of scientific data regarding their use on commercial canola cultivars. For this reason a glasshouse study was conducted to determine the potential of PGRs as anti-lodging agents in canola by measuring their effects on the agronomic and quality characteristics of two different canola cultivars.

3.2. Materials and methods

3.2.1. Experimental site, design and layout

3.2.1.1. Experimental site

This study was conducted under controlled glasshouse conditions at the Department of Agronomy at Stellenbosch University, Western Cape, South Africa.

3.2.1.2. Experimental layout and design

During 2013 a pot trial was conducted in a glasshouse under temperature-controlled conditions (15/10˚C day/night). Due to temperature variation the experimental area was divided into five blocks (replications), each consisting out of 18 pots arranged in a two-way randomized complete block design. The factorial combination comprised of two cultivars of spring canola and four PGR treatments (including an untreated control). One pot represented an experimental unit for each of the eight treatment combinations. Provision has been made for three sampling dates, but because the first sampling was done before the PGR treatments were applied only ten plants (one per cultivar per block) were allotted to this sampling date.

3.2.2. Agronomical practices

3.2.2.1. Pots and growing medium

A total of 90 pots (3 L plastic bags) were used, *viz*. 45 pots for canola cultivar Hyola 555TT and 43C80 respectively. Since coarse sand is low in essential nutrients, it has been extensively used as growing medium for hydroponic trials at Stellenbosch University. After each pot was filled with growing medium (±2 cm below the brim), four drainage holes were spaciously punched in the bottom of each pot. These drainage holes ensured approximately 10% drainage, thus preventing water stress and the build-up of salts in the growing medium.

3.2.2.2. Seed placement and irrigation schedule

On 6 May 2013 four canola seeds were spaciously hand sown pot^{-1}, approximately 1.5 cm deep around each dripper. Growing plants hydroponically, drip irrigation with one outlet (dripper) pot^{-1} was used and controlled by an electric irrigation controller. After planting, each pot was saturated with municipality tap water and kept moist until seedlings appeared. As soon as seedlings started to appear a standard Steiner solution with a nitrate value of 8 me L^{-1} was balanced and used (EC 0.5 mS cm^{-1}), ensuring optimum plant production. While the EC (electrical conductivity) was gradually increased, preventing the newly developed roots from burning, the quantity and frequency of irrigation water were adjusted according to the different growth stages. Plants were irrigated on a daily basis, to prevent water stress and the build-up of salts in the growing medium. At three weeks after emerging, seedlings were

thinned to one vigorous growing plant pot^{-1} in order to minimize differences between plants.

3.2.2.3. Treatments

Treatments were made up of two canola cultivars, Hyola 555TT (TT) and 43C80 (CL) sprayed with three PGRs; Kelpak®, CeCeCe® 750, Moddus® 250 EC and a control (untreated). On 15 July 2013 (70 days after planting) treatments were applied at growth stage 3.1, when the first flower buds became visible in the centre of the leaf rosette (Harper and Berkenkamp 1975). Plant growth regulators were applied at the following dosages in combination with water and wetting agent, Foliwett® 900 if required:

Chlormequat chloride (750 g L^{-1}) applied as CeCeCe® 750 at 5.83 mL L^{-1} water (2.1 L ha^{-1}); trinexapac-ethyl (250 g L^{-1}) applied as Moddus® 250 EC at 1.67 mL L^{-1} water (0.4 L ha^{-1}); and Kelpak® (11.0 mg L^{-1} auxins and 0.031 mg L^{-1} cytokinins from *Ecklonia maxima*) applied at 4.17 mL L^{-1} water (2.0 L ha^{-1}) in combination with Foliwett® 900 (0.06 mL L^{-1} water) (see PGR labels). All PGR spray treatments were done by an automated cabinet sprayer with a moving boom fitted with a flat fan nozzle at a pressure of two bar ensuring a water delivery of 100 L ha^{-1}.

3.2.3. Measurements and analysis

By monitoring growth stages and measuring different plant parameters on different sampling times, the morphological and physiological impact of PGR treatments on growth was determined. When more than 80% of plants reached the following pre-determined growth stages, plants were hand sampled and measured.

The first sampling was done at 70 days after planting, before treatments were applied. Five plants were sampled cultivar^{-1} and the following plant parameters were measured plant^{-1}:

Growth stage using the revised growth-stage key for *B. campestris* and *B. napus* by Harper and Berkenkamp (1975); leaf area plant^{-1} (cm^2) using a LI-3100 leaf area meter; plant height (mm) from the soil surface up to the highest point of the canola plant; lower node diameter (mm); and above ground dry mass (incl. stem, leaves, flowers and pods) (g plant^{-1}). Above ground dry mass plant^{-1} was determined after being dried in an oven at ±75˚C for 72 h in paper bags.

The first sampling was only done to make sure that no differences in plant growth exist before treatments were applied and because no significant differences were noted these results will not be shown or discussed.

The second sampling (93 days after planting) was done during full flowering (growth stage 4.2 according to Harper and Berkenkamp 1975), while the third and final sampling (114 days after planting) was done at start of lower pod filling (growth stage 4.3 according to Harper and Berkenkamp 1975). After sampling five plants treatment^{-1}, all measurements done during the first sampling along with the number of flowers and pods plant^{-1} were measured during the second and third sampling time.

3.2.4. Statistical analysis

An appropriate analysis of variance (ANOVA) was performed, using STATISTICA software, version 12®. The Bonferroni test's least significant difference (LSD) values were calculated at the 5% probability level to facilitate comparison between treatment means.

3.3. Results

Plant growth was generally poor, in spite of the application of a balanced nutrient solution, due to the use of unfertile coarse sand as a growth medium. This poor plant growth might hamper responses to treatments applied and for this reason clear trends were also considered.

3.3.1. Sampling at 93 days after planting

3.3.1.1. Effect of plant growth regulators on vegetative growth

Growth stage

The response of cultivars Hyola 555TT and 43C80 to plant growth regulator treatments are presented in Table 3.1. Instead of mean values, mode values (the value that appears most often in a set of data) had been used to describe the effect of cultivars and PGR treatments on the growth stage at 93 days after planting (Table 3.1), because of the subdivided numbering system used to determine the canola growth stages (Harper and Berkenkamp 1975).

Table 3.1: Growth stage of two canola cultivars as affected by plant growth regulator treatments at 93 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mode
KP	4.2	4.3	4.2
CCC	3.3	4.3	4.3
M	4.2	4.2	4.2
C	4.2	4.3	4.3
Mode	4.2	4.3	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control.

At 93 days after planting (23 days after treatment), cultivar 43C80 already reached growth stage 4.3 on mode, compared to growth stage 4.2 of Hyola 555TT (Table3.1) The PGR treatment CeCeCe® 750 tend to inhibit the growth stage development of Hyola 555TT plants, but little effect was shown on cultivar 43C80 or across cultivars on mode.

Plant height

At 93 days after planting, plants of cultivar 43C80, with a mean plant height of 572.9 mm, were significantly taller than plants of cultivar Hyola 555TT, with a mean plant height of 302.1 mm (Table 3.2). Plant growth regulator treatments had a significant effect on the height of Hyola 555TT plants, as plants treated with Moddus® 250 EC were significantly shorter than Kelpak® treated plants. Although not significant, 43C80 plants treated with Moddus® 250 EC also tend to be shorter than plants from the control or plants treated with CeCeCe® 750. On average, treatment with Moddus® 250 EC tend to reduce canola plant height by almost 24% when compared to the control, while treatment with CeCeCe® 750 or Kelpak® seemed to have very little or no effect. However, differences between mean values were not significant.

Table 3.2: Plant heights (mm) of two canola cultivars as affected by plant growth regulator treatments at 93 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	423.4[a]	522.8[a]	473.1[a]
CCC	250.6[ab]	643.6[a]	447.1[a]
M	199.4[b]	518.0[a]	358.7[a]
C	334.8[ab]	607.2[a]	471.0[a]
Mean	302.1[b]	572.9[a]	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Leaf area

The mean leaf area (cm^2 $plant^{-1}$) at 93 days after planting did not differ significantly between canola cultivars Hyola 555TT and 43C80 (Table 3.3). Plant growth regulator treatments increased the leaf area of Hyola 555TT plants, but no significant differences were shown for 43C80 plants. The largest leaf area of Hyola 555TT plants (217.6 cm^2 $plant^{-1}$) was obtained after applying CeCeCe® 750, while control plants showed the smallest leaf area of 151.7 cm^2 $plant^{-1}$. Although the leaf area of Hyola 555TT plants treated with Kelpak® and Moddus® 250 EC also tend to be larger than the control, differences were not significant. Because PGR treatments did not affect the leaf area of 43C80 plants, mean treatment values did not differ significantly, but the leaf area of the control plants tend to be smaller.

Table 3.3: Leaf area (cm^2 $plant^{-1}$) of two canola cultivars as affected by plant growth regulator treatments at 93 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	203.7[ab]	168.9[a]	186.3[a]
CCC	217.6[a]	151.8[a]	184.7[a]
M	182.0[ab]	165.9[a]	173.9[a]
C	151.7[b]	181.4[a]	166.6[a]
Mean	188.8[a]	167.0[a]	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Lower node diameter

The lower node diameter of the canola cultivars, Hyola 555TT and 43C80 at 93 days after planting did not differ significantly (Table 3.4). Plant growth regulator treatments did affect the lower node diameter of both, but cultivars responded differently. Control plants of Hyola 555TT (5.4 mm) showed the smallest lower node diameter and CeCeCe® 750 (7.1 mm) and Moddus® 250 EC (6.9 mm) the largest lower node diameters. However, in the case of the 43C80 cultivar, control plants (6.9 mm) had the largest lower node diameter, while CeCeCe® 750-treated plants (5.4 mm) showed the smallest lower node diameter. Because the two cultivars tested showed different responses, the mean treatment values did not differ significantly.

Table 3.4: Lower node diameter (mm) of two canola cultivars as affected by plant growth regulator treatments at 93 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	5.8[ab]	6.2[ab]	6.0[a]
CCC	7.1[a]	5.4[b]	6.3[a]
M	6.9[a]	5.8[ab]	6.4[a]
C	5.4[b]	6.9[a]	6.2[a]
Mean	6.3[a]	6.1[a]	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Above ground dry mass

The above ground dry mass (leaves and stems) of canola cultivars Hyola 555TT and 43C80 (Table 3.5), did not differ significantly at 93 days after planting. Dry mass of Hyola 555TT plants were significantly affected by the application of PGRs. The highest dry mass (2.5 g plant^{-1}) was produced by plants treated with Kelpak® and CeCeCe® 750, while the lowest dry mass of 1.8 g plant^{-1} was produced by control plants. Dry mass of 43C80 plants were not significantly affected due to the treatments applied, but PGR-application tend to result in lower plant dry mass over the control. Because of this difference in cultivar response, mean treatment values did not show significant differences.

Table 3.5: Above ground dry mass (g plant^{-1}) of two canola cultivars as affected by plant growth regulator treatments at 93 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	2.5^{a}	2.4^{a}	2.5^{a}
CCC	2.5^{a}	2.5^{a}	2.5^{a}
M	2.3^{ab}	2.3^{a}	2.3^{a}
C	1.8^{b}	2.9^{a}	2.4^{a}
Mean	2.3^{a}	2.5^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

3.3.1.2. Effect of plant growth regulators on reproductive growth

Flower numbers

Even though the 43C80 cultivar showed a larger number of flowers plant^{-1} at 93 days after planting, the cultivars mean values did not differ significantly (Table 3.6). Plant growth regulator treatments did not have a significant effect in any of the cultivars tested, but the treatment with Moddus® 250 EC tended to result in the largest number of flowers plant^{-1} in both cultivars, whereas the control plants tended to produce the smallest number of flowers plant^{-1}.

Table 3.6: Number of flowers plant^{-1} of two canola cultivars as affected by plant growth regulator treatments at 93 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	36.2^{a}	30.0^{a}	33.1^{a}
CCC	30.4^{a}	33.0^{a}	31.7^{a}
M	36.8^{a}	42.6^{a}	39.7^{a}
C	27.4^{a}	28.8^{a}	28.1^{a}
Mean	32.7^{a}	33.6^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Pod numbers plant^{-1}

Because some PGR treatments tend to slow down the growth stage development of Hyola 555TT (Table 3.1), cultivar 43C80 showed a larger number of pods plant^{-1} than Hyola 555TT at 93 days after planting (Table 3.7). No significant differences due to PGR treatment were shown for Hyola 555TT plants, but 43C80 plants treated with CeCeCe® 750 produced a significant larger number of pods plant^{-1} than Kelpak®. However plants (43C80) of the control and Moddus® 250 EC treatments did not differ significantly from that of Kelpak® or CeCeCe® 750.

Table 3.7: Number of pods plant^{-1} of two canola cultivars as affected by plant growth regulator treatments at 93 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	3.4^{a}	4.6^{b}	4.0^{a}
CCC	1.4^{a}	15.2^{a}	8.3^{a}
M	3.4^{a}	10.8^{ab}	7.1^{a}
C	0.0^{a}	12.8^{ab}	6.4^{a}
Mean	2.1^{b}	10.9^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

3.3.2. Sampling at 114 days after planting

3.3.2.1. Effect of plant growth regulators on vegetative growth

Growth stage

At 114 days after planting (44 days after treatment) both cultivars reached growth stage 4.3 on mode (the value that appears most often in a set of data) and did not show any differences due to the PGR treatments (data not shown).

Plant height

At 114 days after planting, plants of cultivar 43C80, with a mean plant height of 987.0 mm, were significantly taller than plants of cultivar Hyola 555TT, with a mean plant height of 744.3 mm (Table 3.8). No significant cultivar by treatment interaction or PGR treatment main effect was shown, but the plants of both cultivars treated with CeCeCe® 750 or Moddus® 250 EC tended to be shorter than the control plants. On average the CeCeCe® 750 and Moddus® 250 EC treatments tended to reduce the plant height with about 17% and 16% when compared to the control. Nevertheless, differences between mean values were not significant.

Table 3.8: Plant height (mm) of two canola cultivars as affected by plant growth regulator treatments at 114 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	781.4[a]	966.6[a]	874.0[a]
CCC	682.2[a]	927.8[a]	805.0[a]
M	685.0[a]	948.0[a]	816.5[a]
C	828.6[a]	1105.4[a]	967.0[a]
Mean	744.3[b]	987.0[a]	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Leaf area

At 114 days after planting, cultivar Hyola 555TT had a significantly larger leaf area (cm^2 $plant^{-1}$) than cultivar 43C80 (Table 3.9). Although no significant differences due to the PGR treatments were shown at this stage, plants of both cultivars treated with

PGRs tended to have smaller leaf areas compared to that of the control. On average, treatment with Moddus® 250 EC, CeCeCe® 750 and Kelpak® tended to reduce the leaf area plant^{-1} by approximately 35%, 28% and 27% when compared to the control.

Table 3.9: Leaf area (cm^2 plant^{-1}) of two canola cultivars as affected by plant growth regulator treatments at 114 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	256.2^a	45.5^a	150.8^a
CCC	215.3^a	80.2^a	147.7^a
M	217.4^a	49.1^a	133.2^a
C	316.5^a	95.8^a	206.2^a
Mean	251.4^a	67.6^b	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Lower node diameter

At 114 days after planting, the lower node diameter of the Hyola 555TT and 43C80 canola cultivars did not differ significantly (Table 3.10). The PGR treatments also did not have a significant effect, but PGR-treated 43C80 plants tended to have smaller lower node diameters than control plants. In contrast to this, Moddus® 250 EC tended to increase the lower node diameter in the case of Hyola 555TT plants.

Table 3.10: Lower node diameter (mm) of two canola cultivars as affected by plant growth regulator treatments at 114 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	6.6[a]	5.4[a]	6.0[a]
CCC	6.2[a]	5.6[a]	5.9[a]
M	7.0[a]	5.4[a]	6.2[a]
C	6.4[a]	6.4[a]	6.4[a]
Mean	6.6[a]	5.7[a]	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Above ground dry mass

With above ground dry mass values of 3.6 and 3.2 g plant^{-1} respectively at 114 days after planting, the canola cultivars Hyola 555TT and 43C80 did not differ significantly (Table 3.11). The application of PGRs significantly affected both Hyola 555TT and 43C80 plants, but the cultivars responded differently. At 114 days after planting Hyola 555TT control plants, showed the highest above ground dry mass of 4.5 g plant^{-1}, while dry mass of plants treated with either CeCeCe® 750 or Kelpak® were significantly reduced (3.0 and 3.3 g plant^{-1} respectively). Although Hyola 555TT plants treated with Moddus® 250 EC tend to have a smaller above ground dry mass than the control plants, differences were not significant. Control 43C80 plants also had the highest dry mass (4.2 g plant^{-1}), but CeCeCe® 750 and Moddus® 250 EC treated plants produced the lowest above ground dry mass (2.5 and 2.9 g plant^{-1} respectively). Although the above ground dry mass of 43C80 plants treated with Kelpak® also tended to be less than that of the control, differences were not significant. Due to these significant differences in cultivar response, mean treatment values also showed larger values for the control plants.

Table 3.11: Above ground dry mass (g plant^{-1}) of two canola cultivars as affected by plant growth regulator treatments at 114 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	3.3^{b}	3.4^{ab}	3.4^{b}
CCC	3.0^{b}	2.5^{b}	2.7^{b}
M	3.8^{ab}	2.9^{b}	3.3^{b}
C	4.5^{a}	4.2^{a}	4.4^{a}
Mean	3.6^{a}	3.2^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

3.3.2.2. Effect of plant growth regulators on reproductive growth

Flower numbers

At 114 days after planting, PGR treatments did not have any effect on the number of flowers produced by 43C80 plants (Table 3.12). However, PGR treatments did have an effect on the number of flowers produced by Hyola 555TT plants, which on average produced significantly more flowers than 43C80 plants (50.4 flowers plant^{-1} compared to 21.3 flowers plant^{-1}). Hyola 555TT plants treated with Moddus® 250 EC showed the largest number of flowers (68.6 flowers plant^{-1}) and CeCeCe® 750-treated plants the lowest (40.4 flowers plant^{-1}). Although Hyola 555TT control and Kelpak®-treated plants also produced fewer flowers than Moddus® 250 EC-treated plants, differences were not significant. When compared to the control on mean treatment, Moddus® 250 EC tend to increase the number of flowers plant^{-1} by almost 29%.

Table 3.12: Number of flowers plant^{-1} of two canola cultivars as affected by plant growth regulator treatments at 114 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	47.2^{ab}	11.0^{a}	29.1^{a}
CCC	40.4^{b}	29.4^{a}	34.9^{a}
M	68.6^{a}	20.8^{a}	44.7^{a}
C	45.2^{ab}	24.0^{a}	34.6^{a}
Mean	50.4^{a}	21.3^{b}	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Pod numbers plant^{-1}

Although the cultivar, 43C80, tended to produce a larger number of pods plant^{-1} (Table 3.13) at 114 days after planting, the cultivars mean values did not differ significantly. Plant growth regulator treatments had a significant effect on the number of pods produced per Hyola 555TT plant but not to that of 43C80. Hyola 555TT plants treated with Moddus® 250 EC produced the largest number of pods (53.6 pods plant^{-1}), whereas CeCeCe® 750-treated plants produced the lowest number of pods (29.8 pods plant^{-1}). Furthermore Hyola 555TT plants treated with Moddus® 250 EC tended to increase the number of pods plant^{-1} by almost 43%, when compared to the control. According to the treatment mean values in Table 3.13, Moddus® 250 EC-treated plants tended to produce the largest number of pods plant^{-1}, while CeCeCe® 750-treated plants, the lowest. However, as in the case of the 43C80 treatment values these values do not differ significantly.

Table 3.13: Number of pods plant^{-1} of two canola cultivars as affected by plant growth regulator treatments at 114 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	37.0^{ab}	50.8^{a}	43.9^{a}
CCC	29.8^{b}	45.4^{a}	37.6^{a}
M	53.6^{a}	49.4^{a}	51.5^{a}
C	37.6^{ab}	55.4^{a}	46.5^{a}
Mean	39.5^{a}	50.3^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, M = Moddus® 250 EC, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

3.4. Discussion

3.4.1. Cultivars

Differences were recorded between cultivars with regard to some parameters measured and different responses to PGR-treatments were also noticed. These results may indicate that cultivars may respond differently to PGR treatments.

3.4.2. Kelpak®

The high auxin (11.0 mg L^{-1}): cytokinin (0.031 mg L^{-1}) ratio of Kelpak® has been reported to promote lateral root formation, which in turn improves top growth and yields on nutrient-stressed okra seedlings and wheat (Nelson and Van Staden 1984; Robertson-Andersson et al. 2006; Khan et al. 2009; Papenfus et al. 2012; Papenfus et al. 2013; Stirk et al. 2014). Supporting these reports, Kelpak® significantly increased the above ground dry mass of Hyola 555TT over the control at 93 days after planting. Taylori et al. (1990) and Ferreira and Lourens (2002) reported similar results with Kelpak® on greenhouse-grown *Phaseolus vulgaris* and synthetic cytokinin, benzyladenine on beans. In contrast to this Kelpak® significantly decreased the above ground dry mass of Hyola 555TT and cultivar average when compared to the control at 114 days after planting.

Khan et al. (2009) reported a potential increase in leaf area and plant chlorophyll content using seaweeds and seaweed products. Although not significant, on cultivar average Kelpak® tended to result in taller plants with larger leaf areas plant^{-1} at 93

days after planting, when compared to the control. Bore and Ng´etich (2007) reported similar results with Kelpak® on nursery tea seedlings. Opposing the results of a study on wheat grown under growth chamber conditions by Nelson and van Staden (1984), Kelpak® treatments did not increase the lower node diameter of canola neither at 93 nor at 114 days after planting.

Supporting previous studies done on cereal production (Taylori et al. 1990), Kelpak® did not significantly increase the number of flowers or pod plant^{-1} during the 2013 glasshouse trial done in unfertile coarse sand, when compared to the control in general. This is in contrast with results obtained from earlier studies done on canola and marigold seedlings (Ferreira and Lourens 2002; Khan et al. 2009) whom reported an increase in the number of flowers, seeds per flower head and yield after Kelpak® treatment.

3.4.3. CeCeCe® 750

Contrary to the current study, previous studies on canola (Armstrong and Nicol 1991; Matysiak and Kaczmarek 2013) reported height reduction under the influence of PGR combinations: chlormequat chloride with paclobutrazol and chlorocholine chloride with tebuconazole. In addition, Giridhar and Giri (1997), Sanvicente et al. (1999), Haque et al. (2007), Zhang et al. (2009), Gebre et al. (2010), Spitzer et al. (2011) and Gebre et al. (2012) reported similar results in various crops, applying chlormequat chloride alone or in PGR combinations.

On average CeCeCe® 750 did not appear to increase the strength of the lower node, even though significant contrasting effects of cultivar Hyola 555TT and 43C80 were shown at 93 days after planting. Although this agrees with results obtained by Passam et al. (2008), Zhang et al. (2009) and Gebre et al. (2012), previous studies on winter barley and sunflowers however showed an increase in lodging resistance by thickening the culm wall and stem width after chlormequat chloride treatment (Sanvicente et al. 1999; Spitzer et al. 2011). Whilst the leaf area and above ground dry mass of Hyola 555TT was significantly increased at 93 days after planting, CeCeCe® 750 significantly decreased the above ground dry mass 114 days after planting, compared to the control.

According to the results obtained from the current study done in coarse sand with low nutrient contents, CeCeCe® 750 generally did not have a significant effect on

reproductive growth when compared to the control. Previous studies however showed a delay in the onset of flowering in sunflowers (Spitzer et al. 2011), as well as increased numbers of pods and yield in groundnut plants and common bent grass (Giridhar and Giri 1997; Aamlid et al. 2007), after applying chlormequat chloride.

3.4.4. Moddus® 250 EC

In accordance to a previous study done on canola (*B. napus* L.), treated with "Moddus" 222.0 g L^{-1} trinexapac applied at 0.5 L ha^{-1} (Ijaz and Honermeier 2012), the application of Moddus® 250 EC tends to reduce height of canola plants during the 2013 glasshouse trial. In addition, Ijaz and Honermeier (2012) obtained the best control of lodging, applying "Moddus" either individually or in combination with a strobilurin fungicide. However, unlike previous cereal-studies done on trinexapac-ethyl alone or in combination with chlormequat (CCC) (Matysiak 2006; Li et al. 2011; Wiersma et al. 2011), Moddus® 250 EC did not significantly reduce plant height over the control in the present study where plants did not grow vigorously due to the use of unfertile coarse sand.

Moddus® 250 EC significantly increased the lower node diameter, of Hyola 555TT at 93 days after planting, but on average, results oppose previous studies (Matysiak 2006; Espindula et al. 2009; Wiersma et al. 2011), as the lower node diameter and leaf area $plant^{-1}$ remained unaffected when compared to the control. According to McCann and Huang (2007) and Wiersma et al. (2011) trinexapac-ethyl application may result in enhanced lignin accumulation in culm cells and chlorophyll content, thereby improving lodging resistance along with photosynthesis activity. Supporting previous studies (Matysiak 2006) at 114 days after planting, Moddus® 250 EC significantly reduce the above ground dry mass of cultivar 43C80 and cultivar average, when compared to the control.

Although Moddus® 250 EC generally did not have a significant effect on reproductive growth when compared to the control; the number of Hyola 555TT flowers- and pods $plant^{-1}$ was significantly increased over CeCeCe® 750 at 114 days after planting. According to Espindula et al. (2009) previous reports of increased yields (Matysiak 2006; Rolston et al. 2010) may be ascribed to morphological changes in the plants architecture, induced by trinexapac-ethyl.

3.5. Conclusion

Although no significant differences in plant height was recorded during the 2013 glasshouse trial with canola conducted in unfertile coarse sand, on average Moddus® 250 EC and CeCeCe® 750 tended to reduce plant height when compared to the control. With the exception of above ground dry mass, significantly reduced by all PGRs at 114 days after planting, PGRs generally did not have a significant effect on vegetative growth when compared to the control, but this may be due to the poor plant growth experienced in the coarse sand used as a growth medium. In general the reproductive growth parameters were not significantly influenced by any of the PGRs tested, when compared to the control. Since results varied between canola cultivar Hyola 555TT and 43C80, it can be concluded that PGRs response may be cultivar specific.

Since only Kelpak® is currently registered for use on canola in South Africa, it is possible that dosage rates and application protocols used for Moddus® 250 EC and CeCeCe® 750 were not necessarily optimal for use in canola. It is recommended that further research including field trials need to be done as PGRs response may be depended on the cultivar, application timing and rates (Matysiak 2006; Wiersma et al. 2011). Moreover, economic analysis needs to be conducted to determine the cost benefit ratio of PGR usage.

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Chapter 4

Efficacy of anti-lodging plant growth regulators on growth and yield of glasshouse grown canola (*Brassica napus* L.) under optimum growth conditions

4.1. Introduction

Canola, *Brassica napus* L. (Brassicaceae), is well adapted to marginal areas of temperate regions (Mosiane et al. 2003), producing a yield of 112 014 tons in South Africa on 72 165 ha during 2013 (Crop estimates 2014). High fertility and irrigation practices are of major importance to sustain or improve the production of canola in South Africa; however, these practices can produce bulky crops prone to lodging (Armstrong and Nicol 1991). Lodging is a constraining factor in terms of production, leading to the reduction in supply of assimilates, sprouting, harvesting efficiency, grain-filling, -quality and -yield, while enhancing disease severity (Armstrong and Nicol 1991; Ramburan and Greenfield 2007; Gebre et al. 2012).

Recent scientific reports on intensively grown cereals have shown that plant growth regulators (PGRs) can successfully be used to reduce plant height, increase lodging resistance, and thus maintaining grain yield (Gebre et al. 2010; Wiersma et al. 2011). This again is in accordance with results obtained by Armstrong and Nicol (1991) on canola in Australia, however, in South Africa there is no detailed information on possibilities for using anti-lodging PGRs on commercial canola cultivars. In an earlier trial different PGRs applied to poorly grown canola plants due to the use of unfertile coarse sand as a growth medium did not show large responses. Because these poor responses may be due to the plants not growing vigorously a second glasshouse trial was conducted in a mixture of coarse sand and compost to test the effect of PGRs as anti-lodging agents under optimal growing conditions by evaluating their effects on the agronomic and quality characteristics of two different canola cultivars. Identifying the most effective PGRs on specific cultivars, the results of the study will contribute to the knowledge of using PGRs in canola management systems to possibly assist in lodging control in South Africa.

4.2. Materials and methods

4.2.1. Experimental site, design and layout

4.2.1.1. Experimental site

This study was conducted under controlled glasshouse conditions at the Department of Agronomy at Stellenbosch University, Western Cape, South Africa.

4.2.1.2. Experimental layout and design

During 2014 a pot trial was conducted in a glasshouse under 15/10˚C day/night controlled temperatures. Due to temperature variation the experimental area was divided into five blocks, each consisting out of 18 pots. Using a two-way randomized complete block design, factorial combination comprised of two cultivars of spring canola, each treated with three PGR treatments and a control (untreated). One pot represented an experimental unit for each of the eight treatment combinations. Provision has been made for three samplings, of which the first was done before the PGR treatments were applied.

4.2.2. Agronomical practices

4.2.2.1. Pots and growing medium

A total of 90 pots (3 L plastic bags) were used for each trial, *viz*. 45 pots for canola cultivar Hyola 555TT and 43C80 respectively. Since coarse sand resulted in poor plant growth during previous hydroponic trials (see Chapter 3), the growing medium was replaced with a 1:1 combination of coarse sand and compost. After filling each pot with growing medium (±2 cm below the brim), four drainage holes were spaciously punched in the bottom of each pot. These drainage holes ensured approximately 10% drainage, thus preventing water stress and the build-up of salts in the growing medium.

4.2.2.2. Seed placement and irrigation schedule

Growing plants hydroponically, drip irrigation with one outlet (dripper) pot^{-1} was used and controlled by an electric irrigation controller. On 17 January 2014 four canola seeds were spaciously hand sown pot^{-1}, 1.5 cm deep around each dripper. The pots were then saturated with municipality tap water and kept moist. As soon as seedlings

started to appear a standard Steiner solution with a nitrate value of 8 me L^{-1} was balanced and used (EC 0.5 mS cm^{-1}), ensuring optimum plant production. Along with the EC (electrical conductivity), the quantity and frequency of irrigation water was gradually increased according to the different growth stages. Plants were regularly irrigated to prevent water stress and build-up of salts in the growing medium. During the first three weeks after emerging, seedlings were thinned to one plant pot^{-1}.

4.2.2.3. Treatments

On the 24th of February 2014 (38 days after planting) treatments were applied at growth stage 3.1, when the first flower buds became visible in the centre of the leaf rosette (Harper and Berkenkamp 1975). Treatments were made up of two cultivars of spring canola, Hyola 555TT (TT) and 43C80 (CL) sprayed with three PGR treatments; Primo MAXX®, CeCeCe® 750, Kelpak® and a control (untreated). The following dosages were applied in combination with water and wetting agent Foliwett® 900 (0.06 mL L^{-1} water):

Trinexapac-ethyl (120 g L^{-1}) applied as Primo MAXX® at 11.11 mL L^{-1} water (4.0 L ha^{-1}); chlormequat chloride (750 g L^{-1}) applied as CeCeCe® 750 at 5.83 mL L^{-1} water (2.1 L ha^{-1}); and Kelpak® (11.0 mg L^{-1} auxins and 0.031 mg L^{-1} cytokinins from *Ecklonia maxima*) at 4.17 mL L^{-1} water (2.0 L ha^{-1}) were applied. Treatments were performed using an automated cabinet sprayer with a moving boom fitted with a flat fan nozzle operator, that deliver 100 L of water ha^{-1} at a pressure of 2 bars.

4.2.3. Measurements and analysis

By monitoring growth stages and measuring different plant parameters on different sampling times, the morphological and physiological impact on growth was determined. After more than 80% of plants reached the pre-determined favoured growth stage, plants were hand sampled and measured.

On the 38th day after planting, the first sampling was done before treatments were applied. Five plants were sampled $cultivar^{-1}$ and the following plant parameters were measured:

Growth stage using the revised growth-stage key for *B. campestris* and *B. napus* by Harper and Berkenkamp (1975); leaf area $plant^{-1}$ (cm^2) using a LI-3100 leaf area meter; plant height (mm) from the soil surface up to the highest point of the canola

plant; lower node diameter (mm); and above ground dry mass (incl. stem, leaves, flowers and pods) (g plant^{-1}). Above ground dry mass plant^{-1} was determined after being dried in an oven at ±75˚C for 72 h in paper bags.

The first sampling was only done to make sure that no differences in plant growth exist before treatments were applied and because no significant differences were noted these results will not be shown or discussed.

The second sampling was done at approximately 55 days after planting (17 days after treatment) during full flowering (growth stage 4.2 according to Harper and Berkenkamp 1975). Five plants were sampled treatment^{-1}. In addition to the measurements done during the first sampling number of flowers and pods plant^{-1} were taken respectively. However at 55 days after planting, exceptionally few pods have been formed and no significant differences due to cultivars or PGR treatments were recorded (data not shown).

The third and final sampling took place when seeds in the lower pods became green-brown mottled (growth stage 5.3 according to Harper and Berkenkamp 1975) at approximately 136 days after planting (98 days after treatment). After sampling five plants treatment^{-1}, all measurements done during the second sampling were repeated along with the following plant parameters:

Flower stalk numbers plant^{-1} (number of stalks emerging from main stem); number of pods flower stalk^{-1} (plant^{-1}); pod dry mass (g plant^{-1}) and mass pod^{-1} (mg plant^{-1}). However at 136 days after planting, nearly all plants finished flowering and no significant differences due to cultivars or PGR treatments were recorded (data not shown).

4.2.4. Statistical analysis

An appropriate analysis of variance (ANOVA) was performed, using STATISTICA software, version 12®. The Bonferroni test's least significant difference (LSD) values were calculated at the 5% probability level to facilitate comparison between treatment means.

4.3. Results

Due to the use of a compost and sand mixture as growth medium, plants grew very vigorously which resembles what should be experienced when canola is grown commercially under irrigation with high fertiliser application rates.

4.3.1. Sampling at 55 days after planting (17 days after treatment)

4.3.1.1. Effect of plant growth regulators on vegetative growth

Growth stage

Table 4.1 shows the response of canola cultivars to PGR treatments at 55 days after planting. Due to the subdivided numbering system used to determine the canola growth stages (Harper and Berkenkamp 1975), mode values (the value that appears most often in a set of data) had been used to describe the effect of cultivars and PGR treatments on the growth stage.

Table 4.1: Growth stage of two canola cultivars as affected by plant growth regulator treatments at 55 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mode
KP	4.2	4.3	4.2
CCC	4.3	4.2	4.3
PM	4.2	4.2	4.2
C	4.2	4.2	4.2
Mode	4.2	4.2	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control.

At 55 days after planting both cultivars Hyola 555TT and 43C80 reached growth stage 4.2 on mode with very little differences due to the treatments applied (Table 4.1).

Plant height

Although mean plant height (mm) did not differ significantly between canola cultivars, PGR treatments had a significant effect (Table 4.2). At 55 days after planting Primo MAXX® significantly decreased the plant height of cultivar Hyola 555TT, when

compared to CeCeCe® 750. In the case of cultivar 43C80, both Primo MAXX® and CeCeCe® 750 tend to produce the smallest plant height values as they differed significantly from Kelpak®. On average, treatment with Primo MAXX® tends to reduce plant height by almost 29% over the control, while at the same time differing significantly from Kelpak®.

Table 4.2: Plant heights (mm) of two canola cultivars as affected by plant growth regulator treatments at 55 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	622.4^{ab}	687.4^{a}	654.9^{a}
CCC	745.2^{a}	428.0^{b}	586.6^{ab}
PM	492.0^{b}	326.8^{b}	409.4^{b}
C	658.8^{ab}	491.0^{ab}	574.9^{ab}
Mean	629.6^{a}	483.3^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Leaf area

At 55 days after planting the mean leaf area (cm^2 $plant^{-1}$) did not differ significantly between canola cultivars Hyola 555TT and 43C80 (Table 4.3) and PGR treatments also had no significant effect.

Table 4.3: Leaf area (cm^2 $plant^{-1}$) of two canola cultivars as affected by plant growth regulator treatments at 55 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	1369.2^{a}	1517.2^{a}	1443.2^{a}
CCC	1467.7^{a}	1572.2^{a}	1520.0^{a}
PM	1250.0^{a}	1778.4^{a}	1514.2^{a}
C	1524.5^{a}	1549.7^{a}	1537.1^{a}
Mean	1402.9^{a}	1604.4^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Lower node diameter

At 55 days after planting the mean lower node diameter of cultivar Hyola 555TT (12.8 mm) were significantly smaller than cultivar 43C80 (17.3 mm) (Table 4.4), but PGR treatments did not have any significant effect.

Table 4.4: Lower node diameter (mm) of two canola cultivars as affected by plant growth regulator treatments at 55 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	13.4[a]	17.6[a]	15.5[a]
CCC	14.4[a]	17.6[a]	16.0[a]
PM	11.4[a]	16.4[a]	13.9[a]
C	12.0[a]	17.4[a]	14.7[a]
Mean	12.8[b]	17.3[a]	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Above ground dry mass

With mean above ground dry mass values of 14.6 and 14.1 g plant^{-1} respectively, the canola cultivars Hyola 555TT and 43C80 did not differ significantly at 55 days after planting (Table 4.5). The application of PGRs significantly affected both Hyola 555TT and 43C80 plants as plants treated with Primo MAXX® showed a smaller above ground dry mass compared to other treatments, reducing the average above ground dry mass plant^{-1} by approximately 17% when compared to the control.

Table 4.5: Above ground dry mass (g plant^{-1}) of two canola cultivars as affected by plant growth regulator treatments at 55 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	14.6[a]	15.4[a]	15.0[a]
CCC	16.1[a]	14.1[a]	15.1[a]
PM	12.7[b]	12.0[b]	12.4[b]
C	14.8[a]	14.9[a]	14.9[a]
Mean	14.6[a]	14.1[a]	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

4.3.1.2. Effect of plant growth regulators on reproductive growth

Flower numbers

At 55 days after planting, canola cultivar Hyola 555TT tended to have more flowers than 43C80, but differences were not significant (Table 4.6). Plant growth regulator treatments did not have a significant effect, although Primo MAXX®, Kelpak® and CeCeCe® 750, on average, increased the number of flowers plant^{-1} by approximately 13%, 19% and 19% respectively, when compared to the control.

Table 4.6: Number of flowers plant^{-1} of two canola cultivars as affected by plant growth regulator treatments at 55 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	112.2[a]	100.0[a]	106.1[a]
CCC	127.8[a]	84.8[a]	106.3[a]
PM	119.8[a]	81.2[a]	100.5[a]
C	90.2[a]	88.2[a]	89.2[a]
Mean	112.5[a]	88.6[a]	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

4.3.2. Sampling at 136 days after planting (98 days after treatment)

4.3.2.1. Effect of plant growth regulators on vegetative growth

Growth stage

At 136 days after planting, both canola cultivars reached growth stage 5.3 on mode and no significant differences due to PGR treatments were shown (data not shown).

Plant height

At 136 days after planting, plants of cultivar 43C80, with a mean plant height of 1399.8 mm, were significantly taller than plants of cultivar Hyola 555TT, with a mean plant height of 1134.9 mm (Table 4.7). Although CeCeCe® 750 and Primo MAXX® tended to reduce the height of Hyola 555TT plants, differences were not significant. In the case of cultivar, 43C80 and on average, Primo MAXX® did result in a significant reduction in plant height when compared to control and Kelpak®-treated plants. When compared to the control, Primo MAXX® reduced the plant height by almost 25% on average.

Table 4.7: Plant height (mm) of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	1276.0[a]	1485.0[a]	1380.5[a]
CCC	993.6[a]	1492.0[a]	1242.8[ab]
PM	988.0[a]	1108.0[b]	1048.0[b]
C	1282.0[a]	1514.0[a]	1398.0[a]
Mean	1134.9[b]	1399.8[a]	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Lower node diameter

At 136 days after planting, cultivar 43C80 had a lower node diameter of 21.1 mm, compared to 16.0 mm of cultivar Hyola 555TT (Table 4.8). Although all PGR treatments tend to increase the lower node diameter of 43C80 plants, only Primo MAXX® treated plants had a significant larger lower node diameter when compared

to the control. In contrast Hyola 555TT plants did not show any significant differences in lower node diameter due to treatments applied.

Table 4.8: Lower node diameter (mm) of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	16.2[a]	20.8[ab]	18.5[a]
CCC	14.8[a]	21.6[ab]	18.2[a]
PM	16.4[a]	23.4[a]	19.9[a]
C	16.6[a]	18.4[b]	17.5[a]
Mean	16.0[b]	21.1[a]	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Flower stalk numbers

The mean number of flower stalks plant^{-1} at 136 days after planting did not differ significantly between canola cultivars Hyola 555TT and 43C80 (Table 4.9). No significant cultivar by treatment interaction was shown and plants of both cultivars treated with PGRs tended to have a larger number of flower stalks plant^{-1} when compared to the control. On average, treatment with Primo MAXX® significantly increased the number of flower stalks plant^{-1} by approximately 28% when compared to the control, but no significant differences due to CeCeCe® 750 and Kelpak® treatments were recorded.

Table 4.9: Number of flower stalks plant^{-1} of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	9.8^{a}	8.8^{a}	9.3^{ab}
CCC	9.0^{a}	8.8^{a}	8.9^{ab}
PM	9.8^{a}	10.2^{a}	10.0^{a}
C	7.6^{a}	8.0^{a}	7.8^{b}
Mean	9.1^{a}	9.0^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Above ground dry mass

The mean above ground dry mass of canola cultivars Hyola 555TT and 43C80 did not differ significantly at 136 days after planting (Table 4.10). However, PGR treatments significantly affected both Hyola 555TT and 43C80 plants, but cultivars responded differently. Primo MAXX® reduced the above ground dry mass of 43C80 plants significantly when compared to the control. On average, plants treated with Primo MAXX® significantly reduced the above ground dry mass when compared to the control and Kelpak®-treated plants.

Table 4.10: Above ground dry mass (g plant^{-1}) of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	149.0^{a}	151.3^{ab}	150.2^{a}
CCC	123.4^{ab}	150.2^{ab}	136.8^{ab}
PM	110.8^{b}	126.0^{b}	118.4^{b}
C	139.6^{ab}	160.1^{a}	149.9^{a}
Mean	130.7^{a}	146.9^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

4.3.2.2. Effect of plant growth regulators on reproductive growth

Pod numbers plant^{-1}

At 136 days after planting no significant differences in number of pods plant^{-1} were recorded between Hyola 555TT and 43C80 plants (table 4.11) and PGR treatments also did not have any significant effect on the cultivars. Although not significant, on average treatment with CeCeCe® 750, Primo MAXX® and Kelpak® increased the number of pods plant^{-1} by approximately 15%, 21% and 41% respectively, when compared to the control.

Table 4.11: Number of pods plant^{-1} of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	2120.0^{a}	1320.0^{a}	1720.0^{a}
CCC	1396.0^{a}	1426.0^{a}	1411.0^{a}
PM	1496.0^{a}	1464.0^{a}	1480.0^{a}
C	1180.0^{a}	1268.0^{a}	1224.0^{a}
Mean	1548.0^{a}	1369.5^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Pod numbers flower-stalk^{-1}

On the 136th day after planting, cultivars tested and treatments applied had no significant effect on the mean number of pods flower-stalk^{-1} (Table 4.12).

Table 4.12: Pod numbers flower-stalk^{-1} of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	200.4^{a}	150.0^{a}	175.2^{a}
CCC	153.2^{a}	164.8^{a}	159.0^{a}
PM	154.0^{a}	148.0^{a}	151.0^{a}
C	161.8^{a}	158.6^{a}	160.2^{a}
Mean	167.4^{a}	155.4^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Pod dry mass plant^{-1}

With mean pod dry mass values of 63.2 and 79.8 g plant^{-1} respectively (136 days after planting), the canola cultivars Hyola 555TT and 43C80 did not differ significantly (Table 4.13). In the case of cultivar 43C80 and on average for both cultivars, pod dry mass of Primo MAXX®-treated plants was significantly less than that of control plants. Although not significant a similar trend was shown for Hyola 555TT plants.

Table 4.13: Pod dry mass (g plant^{-1}) of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	61.7^{a}	90.3^{ab}	76.0^{ab}
CCC	59.7^{a}	76.8^{ab}	68.2^{ab}
PM	52.8^{a}	60.6^{b}	56.7^{b}
C	78.5^{a}	91.4^{a}	84.9^{a}
Mean	63.2^{a}	79.8^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

Mass pod^{-1}

According to Table 4.14, the mean mass pod^{-1} values of cultivar Hyola 555TT and 43C80 did not differ significantly at 136 days after planting. Plant growth regulator treatments had a significant effect on the mass pod^{-1} produced per 43C80 plant but not to that of Hyola 555TT. Untreated (control) plants of cultivar 43C80 (92.4 mg) recorded the largest mass pod^{-1}, while Primo MAXX® (44.8 mg) significantly reduced the mass pod^{-1} compared to the control. On average, treatment with Primo MAXX® reduced the mean mass pod^{-1} by approximately 47% when compared to the control, while treatment with CeCeCe® 750 or Kelpak® seemed to have very little or no effect.

Table 4.14: Mass pod^{-1} (mg) of two canola cultivars as affected by plant growth regulator treatments at 136 days after planting

Treatment	Cultivar		
	Hyola 555TT	43C80	Mean
KP	48.5^{a}	81.1^{ab}	64.8^{ab}
CCC	55.4^{a}	64.0^{ab}	59.7^{ab}
PM	40.4^{a}	44.8^{b}	42.6^{b}
C	67.6^{a}	92.4^{a}	80.0^{a}
Mean	53.0^{a}	70.6^{a}	

KP = Kelpak®, CCC = CeCeCe® 750, PM = Primo MAXX®, C = Control. *Values in the same column and means followed by the same letter do not differ significantly at p=0.05*

4.4. Discussion

4.4.1. Cultivars

Although differences were recorded between cultivars with regard to vegetative growth and some differences in response to PGR treatments were also noted, trends were not clear. With regards to reproductive growth, no significant differences between cultivars were recorded but cultivar 43C80 seemed to be more responsive to Primo MAXX® treatment than Hyola 555TT as measured in significant lower pod dry mass per plant and mass per pod when compared to control plants.

4.4.2. Kelpak®

Compared to the control and results of various previous studies (Ferreira and Lourens 2002; Khan et al. 2009; Papenfus et al. 2012), the application of Kelpak® did not significantly enhance the overall plant vigour during the 2014 glasshouse trial done on canola under optimal conditions. Although Kelpak® treatments tend to result in taller plants with small leaf areas plant^{-1} throughout the current study; results were not significant when compared to the control. On the contrary, Bore and Ng´etich (2007) and Khan et al. (2009) reported a potential increase in leaf area and plant chlorophyll content using seaweed products including Kelpak®. In contrast to previous studies on wheat grown under growth chamber conditions (Nelson and van Staden 1984), the application of Kelpak® did not significantly increase the lower node diameter of neither cultivar Hyola 555TT nor 43C80. Furthermore Kelpak® treatment did not significantly increase the number of flower stalks plant^{-1} nor the above ground dry mass of vigorous growing plants in the 2014 glasshouse trial, whereas Taylori et al. (1990), Ferreira and Lourens (2002) reported a potential increase in top growth.

Although Kelpak® tend to increase the number of pods plant^{-1} and flower stalks plant^{-1} of Hyola 555TT as well as the means across cultivars when compared to the control, differences were not significant. These results supported previous studies done on cereal production (Taylori et al. 1990), but were in contrast to the results of Ferreira and Lourens (2002) and Khan et al. (2009) who reported an increase in the number of flowers, pods and yield in canola (2 L ha^{-1} Kelpak®, applied at 3- or 5-leaf stages) and marigold seedlings after Kelpak® treatment.

4.4.3. CeCeCe® 750

Differences in plant height between CeCeCe® 750 and control plants were relatively small if any in the glasshouse trial where canola plants were grown vigorously under optimum conditions. This is in contrast to reductions in plant height reported in response to chlormequat chloride alone or in PGR combinations in earlier studies done on various crops, including canola (Armstrong and Nicol 1991; Giridhar and Giri 1997; Sanvicente et al. 1999; Haque et al. 2007; Ramburan and Greenfield 2007; Zhang et al. 2009; Gebre et al. 2010; Spitzer et al. 2011; Gebre et al. 2012; Matysiak and Kaczmarek 2013).

In support to the results obtained by Passam et al. (2008), Zhang et al. (2009) and Gebre et al. (2012), the lower node diameter remained unaffected after CeCeCe® 750 treatment. Generally CeCeCe® 750 did not have a significant effect on leaf area-, above ground dry mass- or on flower stalks plant^{-1}, when compared to the control when applied to vigorously growing plants in this study. Contrary to Giridhar, Giri (1997) and Aamlid et al. (2007), CeCeCe® 750 generally also did not have a significant effect on reproductive growth when compared to the control.

4.4.4. Primo MAXX®

During the 2014 glasshouse trial, Primo MAXX® applied to vigorously growing plants tends to reduced plant height of both Hyola 555TT and 43C80 at 55 days after planting and reduced plant height of cultivar 43C80 as well as cultivar mean significantly when compared to the control at 136 days after planting. Previous reports on plant height in response to trinexapac-ethyl alone or in combination with chlormequat (CCC), varied from no effects in sunflowers (Spitzer et al. 2011), to tendency to reduce height in canola (*B. napus* L.) (Ijaz and Honermeier 2012), to significantly reduced height in cereals (Matysiak 2006; Li et al. 2011; Wiersma et al. 2011). Ijaz and Honermeier (2012) furthermore obtained the best control of lodging in canola, applying “Moddus” either individually or in combination with a strobilurin fungicide.

In support to earlier studies with trinexapac-ethyl (Matysiak 2006; Espindula et al. 2009; Wiersma et al. 2011), Primo MAXX® increased the lower node diameter of cultivar 43C80 significantly at 136 days after planting when compared to the control, but cultivar means of the lower node diameter and leaf area plant^{-1} were not affected. Primo MAXX® applications to vigorously growing plants resulted in a significant decrease in above ground dry mass when compared to the control, which support earlier research (Matysiak 2006).

Although Primo MAXX® did result in a significant increase in the number of flower stalks plant^{-1} at 136 days after planting, the number of pods plant^{-1} as well as pod numbers flowerstalk^{-1} were not affected. Primo MAXX®, however, caused a significant decrease in pod dry mass plant^{-1} and mass pod^{-1} of cultivar 43C80 when compared to the control at 136 days after planting indicating. Although similar trends

were shown for Hyola 555TT differences were not significant, suggesting some differences in cultivar sensitivity.

4.5. Conclusion

Based on the cultivar means obtained during the 2014 glasshouse trial with canola under conditions which represent commercial production under irrigation or in high rainfall areas with high soil fertility levels or fertiliser applications, Primo MAXX® tend to be more efficient than other PGRs tested in reducing plant height at 55 days after treatment and resulted in a significant reduction in plant height at 136 days after planting (98 days after treatment) when compared to the control.

Primo MAXX® also had the largest effect on vegetative growth as shown in a significant reduction in above ground dry mass throughout the trial. Although Primo MAXX® resulted in an increase in the number of flower stalks plant^{-1} and lower node diameter at 136 days after planting when compared to the control pod dry mass plant^{-1} and mass pod^{-1} were reduced. These results may indicate a possible reduction in yield due to the use of Primo MAXX®, but may also be due to an increase in the pod filling stage after a Primo MAXX® application due to the larger number of flower stalks plant^{-1}. Unfortunately all pots in the trial had to be harvested at the same time due to the randomised lay-out of the trial and to prevent losses due to shattering. Harvesting was done when the first pods showed signs of ripening.

Due to results varying between cultivar Hyola 555TT and 43C80, it can be concluded that PGRs response may be cultivar specific.

Apart from Kelpak®, none of the PGRs tested in this study is currently registered for use on canola in South Africa. Since dosage rates and application protocols used for CeCeCe® 750 and Primo MAXX® may not have been optimal for use in canola, further research needs to be done as PGRs response may be dependent on the cultivar, PGR combinations, application timing and rates (Matysiak 2006; Wiersma et al. 2011). Additionally, economic analysis needs to be conducted to determine the cost benefit ratio of PGR usage.

4.6. References

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Chapter 5

Effect of anti-lodging plant growth regulators on growth and yield of canola (*Brassica napus* L.) grown at different localities in the Western Cape Province of South Africa

5.1. Introduction

Canola, *Brassica napus* L. (Brassicaceae) is becoming one of the leading sources of protein and oil worldwide, producing a yield of 112 014 tons in South Africa on 72 165 ha during 2013 (Crop estimates 2014) compared to around 5 000 ha planted during 1994 (Mosiane et al. 2003). In South Africa, successful seed production predominantly depends on high fertility and irrigation practices, however, these practices can produce bulky crops susceptible to lodging (Armstrong and Nicol 1991). Lodging is a critical restriction to the production of canola, decreasing the supply of assimilates, sprouting, harvesting efficiency, grain-filling, -quality and -yield, while enhancing disease severity (Armstrong and Nicol 1991; Ramburan and Greenfield 2007; Gebre et al. 2012).

Thus far plant growth regulators (PGRs) have been successfully utilized in high input cereal management systems to reduce plant height and improve lodging resistance, whilst maintaining grain yield (Gebre et al. 2010; Wiersma et al. 2011). In Australia, Armstrong and Nicol (1991) accordantly reported similar results on canola. However, in South Africa anti-lodging PGRs are at present not used on commercial canola cultivars. Since the response to PGR applications may be affected by growth conditions, the aim of these field trials were to determine the effect of anti-lodging PGR agents on the agronomic and quality characteristics of canola under field conditions at different localities in the Western Cape.

5.2. Materials and methods

5.2.1. Experimental site and soil

Field trials were conducted during 2013 at Langgewens (-33.17˚; 18.42˚; altitude 177 m), Altona (-33.40˚; 18.35˚; altitude 76 m) and Roodebloem (-34.19˚; 19.31˚; altitude 122 m) Research Farms in the Western Cape Province of South Africa. The soil texture of Langgewens and Altona can be described as sandy-loam, and loamy at

Roodebloem (Table 5.1) with pH (KCl) values of 6.1, 5.63 and 5.13 at Langgewens, Altona and Roodebloem respectively which were near optimum for canola production (Anon 2013).

Table 5.1: Physical and chemical properties of the soil at Langgewens-, Altona-, and Roodebloem Research Farms, sampled in the beginning of 2013 growing season and analysed at the laboratories of the Department of Agriculture, Western Cape, using standard procedures

Physical and chemical properties		Locality		
		Langgewens	Altona	Roodebloem
Texture		Sandy-loam	Sandy-loam	Loam
pH	(KCl)	6.10	5.63	5.13
Resistance	(ohm)	1323.33	1106.67	816.67
Ca	(cmol kg^{-1})	3.67	3.19	4.28
Mg		1.37	0.48	1.17
Na	(mg kg^{-1})	33.00	22.33	34.67
K		44.00	107.67	209.33
P		133.00	70.00	46.33
Cu		1.71	0.77	0.96
Zn		0.94	1.03	2.14
Mn		11.04	66.90	177.90
B		0.16	0.12	0.253
S		5.77	3.07	3.32
C	(%)	0.83	0.6	1.5
NH_4-N		0.07	0.05	0.16

5.2.2. Climate

The total monthly rainfall at Langgewens, Altona, and Roodebloem during 2013 (April 1st to October 31st) are shown in Figure 5.1. During 2013 rainfall (mm) received during April and October did not differ much from the long term mean, while less than mean values for the long term rainfall was recorded during May and July. These drier months could have resulted in some degree of water stress during germination and budding to early flowering stage. During the month of June, Altona and

Roodebloem received rainfall above the long term mean, whereas Langgewens received less. The higher than mean values for long term rainfall during August and September 2013, could have resulted in lavish growth and some degree of lodging.

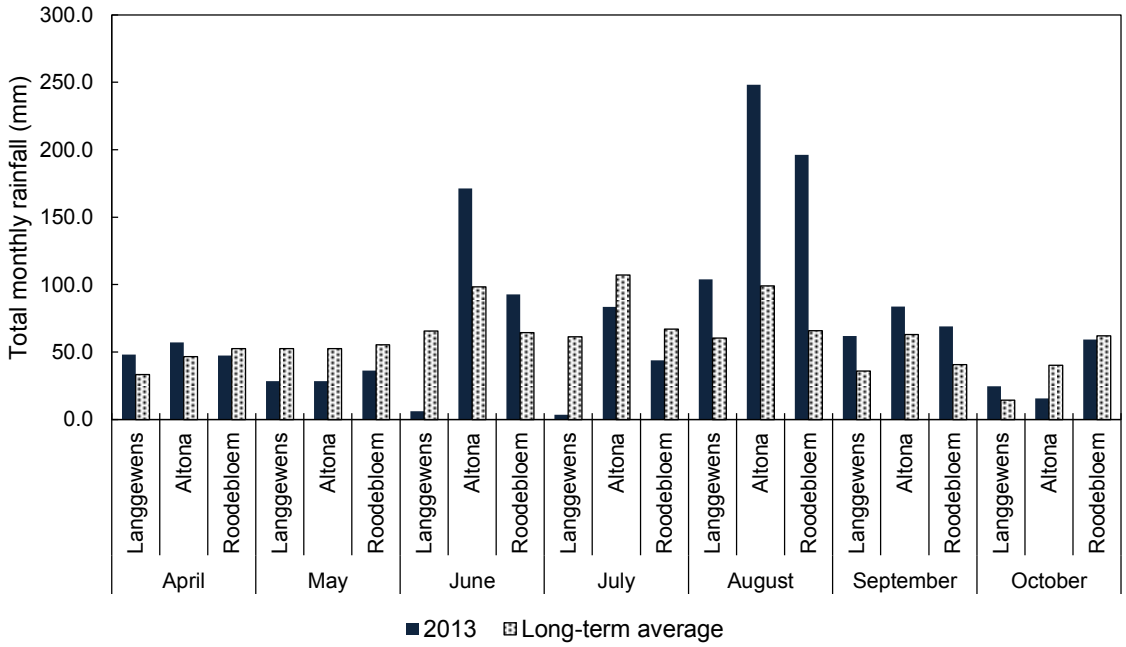

Figure 5.1: The long-term total monthly rainfall (mm) compared to total monthly rainfall for the period April to October 2013, at the Langgewens-, Altona-, and Roodebloem Research Farms (Data from the ARC-ISCW)

Figures 5.2 and 5.3 show the mean daily maximum and minimum temperature (˚C) for the period April 1st to October 31st 2013, at the Langgewens-, Altona-, and Roodebloem Research Farms. During the months of April, June, August, September and October the majority of localities showed mean daily maximum temperatures which were lower than mean long-term values. In contrast to this, mean daily maximum temperatures during May and June were higher than long term mean values at most localities. Mean daily minimum temperatures, during the period April to October were with the exception of July at most of the localities lower than long-term mean values. Although the majority of mean daily maximum and minimum temperatures during 2013 appear to be below long-term means, 2013 temperatures still were within the optimum range for canola production (Anon 2013).

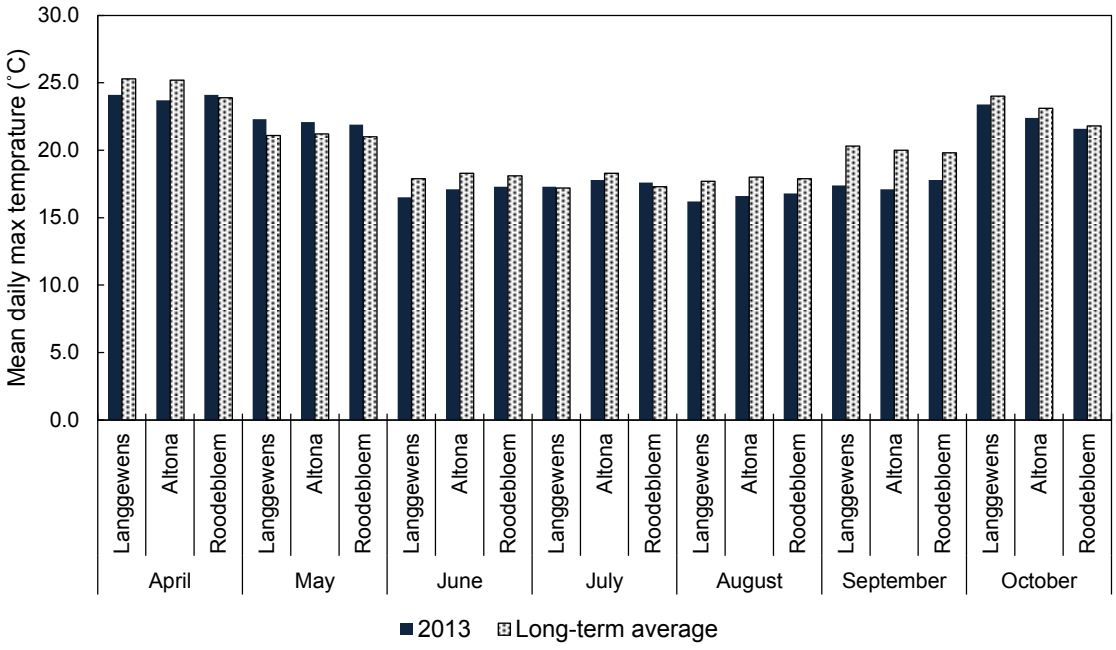

Figure 5.2: The long-term mean daily maximum temperature (˚C) compared to the period, April to October 2013, at the Langgewens-, Altona-, and Roodebloem Research Farms (Data from the ARC-ISCW)

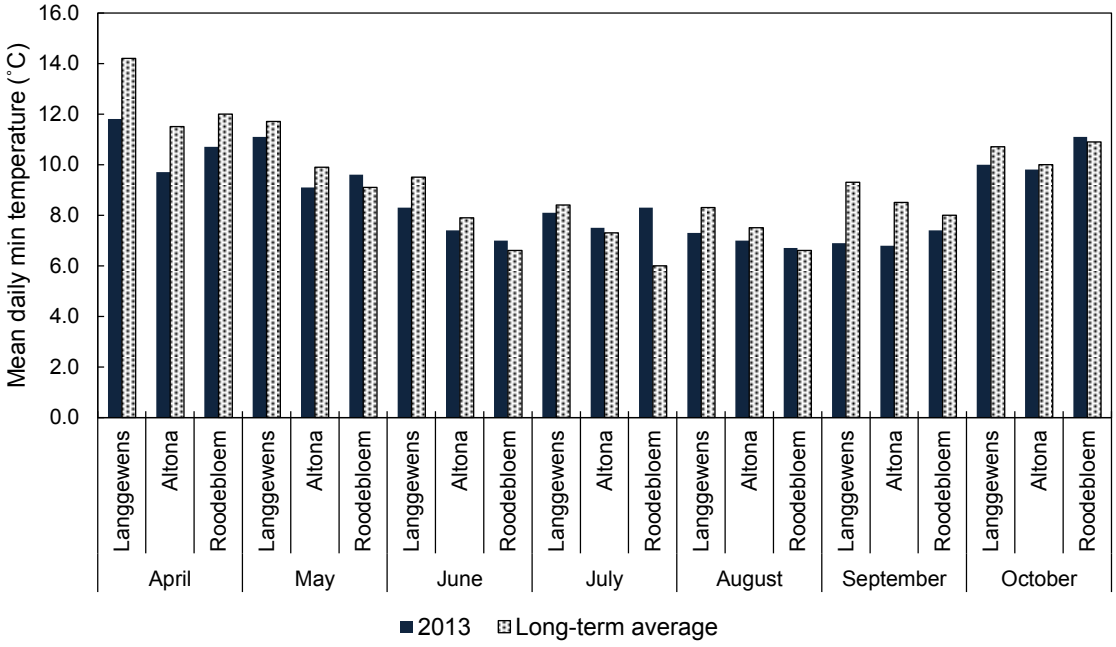

Figure 5.3: The long-term mean daily minimum temperature (˚C) compared to the period April to October 2013, at the Langgewens-, Altona-, and Roodebloem Research Farms (Data from the ARC-ISCW)

5.2.3. Agronomical practices

Spring canola cultivar, Hyola 555TT was planted at Altona, Langgewens and Roodebloem on 6, 7 and 10 May 2013 respectively, using an experimental plot planter. The planting density of 4 kg ha^{-1} resulted in plant populations of 55, 46 and 23 plants m^{-2} at the Langgewens, Altona and Roodebloem Research Farms respectively.

To control insect pests a pesticide containing active ingredient dimetoaat and slug pellets were applied at planting and at seedling stage. To control weeds an herbicide containing the active ingredient atrazine was applied at all localities, an additional application with a grass-weed herbicide was applied at Altona.

To create optimum nutritional conditions, nitrogen was applied as recommended for different production areas and crop rotation systems, while phosphorous, potassium and boron were applied according to the soil analysis.

5.2.4. Treatments, experimental design and experimental layout

Plant growth regulator treatments were applied to Langgewens and Altona on the 22nd of July, while Roodebloem was treated on the 24th of July 2013. Four PGR treatments: Kelpak®, CeCeCe® 750, Moddus® 250 EC and Primo MAXX® and a control (untreated) were used. Following the recommendations on their labels, PGRs were applied at the following dosage rates:

Chlormequat chloride (750 g L^{-1}) applied as CeCeCe® 750 at 2.1 L ha^{-1}; trinexapac-ethyl (250 g L^{-1}) applied as Moddus® 250 EC at 0.4 L ha^{-1}; trinexapac-ethyl (120 g L^{-1}) applied as Primo MAXX® at 4.0 L ha^{-1}; and Kelpak® (11.0 mg L^{-1} auxins and 0.031 mg L^{-1} cytokinins from *Ecklonia maxima*) applied at 2.0 L ha^{-1} in combination with Foliwett® 900 (0.06 mL L^{-1} water). All treatments were applied during growth stage 3.1 (Harper and Berkenkamp 1975), when flower buds became visible at the centre of the leaf rosette. A 20 L knapsack sprayer was used to apply the PGRs at a spraying volume of 200 L ha^{-1}.

A randomized complete block design comprised of five treatments, replicated four times was used. Each experimental unit (4.5 m × 5 m) was subdivided into three sub-plots: Sub-plot 1 was used for the first three plant samplings; while sub-plot 2 and 3 were used for the fourth and final plant sampling respectively.

5.2.5. Measurements

By monitoring and measuring different plant parameters at four sampling times at Langgewens and Altona, the morphological and physiological impact of PGRs on growth was determined. At Roodebloem only the first and final samplings were done.

First sampling

At approximately 76 days after planting, the first sampling was done prior to the application of the PGR treatments at the start of budding and stem elongation (growth stage 3.1 according to Harper and Berkenkamp 1975). Two plants were randomly sampled plot^{-1} to measure:

Growth stage using the revised growth-stage key for *B. campestris* and *B. napus* by Harper and Berkenkamp (1975); leaf area plant^{-1} (cm^2) using a LI-3100 leaf area meter; plant height (mm) from the soil surface up to the highest point of the canola plant; lower node diameter (mm); above ground dry mass (incl. stem, leaves, flowers and pods) (g plant^{-1}); and root dry mass (g plant^{-1}) after being carefully pulled from the wet soil. Above ground and root dry mass plant^{-1} was determined after it has been dried in an oven at ±75˚C for 72 h in paper bags.

Sampling one was only done to make sure that no differences in plant growth exist before treatments were applied and because no significant differences were noted, these results will not be shown or discussed.

Second sampling

The second sampling was done at approximately 110 days after planting (34 days after treatment) during lower pod filling (growth stage 4.3 according to Harper and Berkenkamp 1975). Five plants were randomly sampled plot^{-1}. Measurements done during the first sampling were repeated with the addition of number of flowers and pods plant^{-1} respectively.

Third sampling

During the third sampling, when seeds in lower pods reached their full size and became translucent (growth stage 5.1 according to Harper and Berkenkamp 1975), at approximately 125 days after planting (49 days after treatment) five plants were

again randomly sampled plot^{-1}. As plants already started to ripen, only plant height, number of pods plant^{-1} as well as root and above ground dry mass were determined.

Fourth sampling

The fourth and final sampling took place when all plants ripened (growth stage 5.5 according to Harper and Berkenkamp 1975) at approximately 195 days after planting (119 days after treatment). Plots were harvested with an experimental plot harvester/combiner to determine the grain yield in ton ha^{-1} along with the mass seed^{-1} in mg.

5.2.6. Statistical analysis

An appropriate analysis of variance (ANOVA) was performed, using STATISTICA software, version 12®. The Bonferroni test's least significant difference (LSD) values were calculated at the 5% probability level to facilitate comparison between treatment means.

5.3. Results

No signs of lodging were observed during the present field trials study (2013) done on canola at Langgewens, Altona and Roodebloem. Moreover no significant locality × treatment interaction was found for any of the parameters measured. For this reason only main effects will be discussed.

5.3.1. Sampling at 110 days after planting

5.3.1.1. Effect of plant growth regulators on vegetative growth

Plant height

At 110 days after planting (34 days after treatment), the mean plant height (mm) did not differ significantly between the two localities, Altona and Langgewens (Table 5.2), but PGR treatments had a significant effect (Figure 5.4).

Table 5.2: Plant parameters of canola cultivar Hyola 555TT at Langgewens, Altona and Roodebloem as affected by plant growth regulator treatments at 110, 125 and 195 days after planting

Plant parameters		Locality		
		Langgewens	Altona	Roodebloem
110 Days after planting				
Plant height	(mm)	947.2[a]	995.0[a]	*
Leaf area	(cm^2 $plant^{-1}$)	601.5[a]	547.9[a]	*
Lower node diameter	(mm)	9.8[a]	8.8[b]	*
Above ground dry mass	(g $plant^{-1}$)	11.8[a]	9.9[a]	*
Root dry mass	(g $plant^{-1}$)	1.9[a]	1.7[a]	*
Flower numbers	($plant^{-1}$)	308.8[a]	233.1[b]	*
Pod numbers	($plant^{-1}$)	62.4[a]	32.9[b]	*
125 Days after planting				
Plant height	(mm)	1035.8[a]	1047.6[a]	*
Above ground dry mass	(g $plant^{-1}$)	21.1[a]	17.8[a]	*
Root dry mass	(g $plant^{-1}$)	1.7[a]	1.4[a]	*
Pod numbers	($plant^{-1}$)	212.9[a]	139.8[b]	*
195 Days after planting				
Mass $seed^{-1}$	(mg)	2.7[b]	2.5[c]	3.2[a]
Grain yield	(ton ha^{-1})	1.712[b]	1.911[a]	1.603[b]

*Values in the same row followed by the same letter do not differ significantly at p=0.05; *= data not recorded*

Hyola 555TT plants treated with Kelpak®, with a mean plant height of 1174.8 mm, were significantly taller than plants treated with Primo MAXX®, with a mean plant height of 795.0 mm. On average, treatment with Kelpak® tends to increase canola plant height by almost 24%, while treatment with Primo MAXX® seemed to reduce the plant height with approximately 16% when compared to the control.

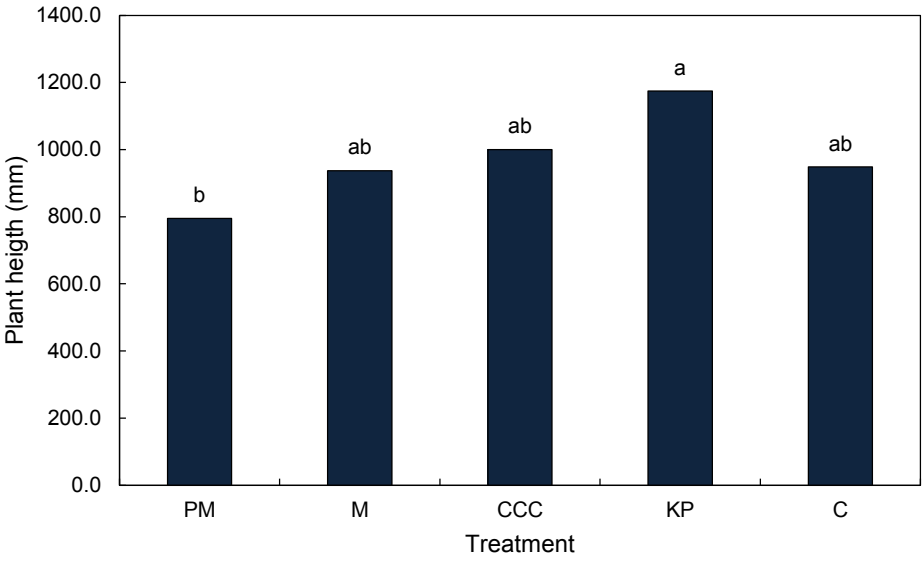

Figure 5.4: Plant heights (mm) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 110 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

Leaf area

At 110 days after planting, the mean leaf area (cm^2 $plant^{-1}$) did not differ significantly between the two localities (Altona and Langgewens) (Table 5.2), nor did it differ between the different treatments (Figure 5.5). Nevertheless at 110 days after planting, all PGR treatments tend to increase the leaf area $plant^{-1}$ when compared to the control. Of the four PGRs Kelpak®-treated Hyola 555TT plants showed the largest leaf area $plant^{-1}$ (720.7 cm^2 $plant^{-1}$), while Moddus® 250 EC-treated plants showed the smallest (512.0 cm^2 $plant^{-1}$).

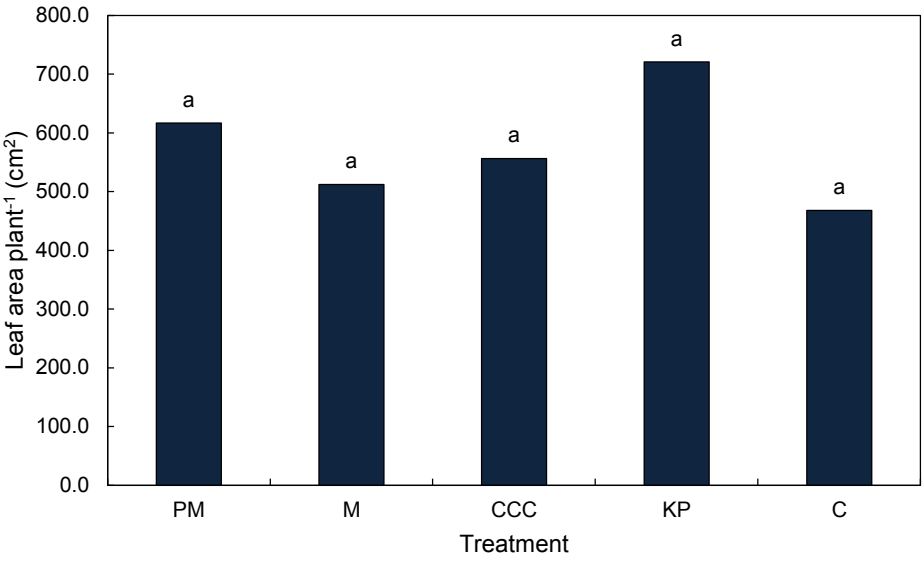

Figure 5.5: Leaf area (cm^2 $plant^{-1}$) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 110 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

Lower node diameter

The mean lower node diameter (mm) at Langgewens (9.8 mm) was significantly larger compared to Altona (8.8 mm) (Table 5.2). The PGR treatments also had a significant effect on the lower node diameter of Hyola 555TT plants (Figure 5.6). At 110 days after planting, all four PGR treatments tend to increase the lower node diameter when compared to the control. Even though the lower node diameter of Kelpak®-treated plants did not differ significantly from the other PGR treatments, it was significantly larger than that of the control. When compared to the control, plants treated with Kelpak® increased the lower node diameter with almost 14%, while treatment with Primo MAXX®, Moddus® 250 EC and CeCeCe® 750 did not have significant effects.

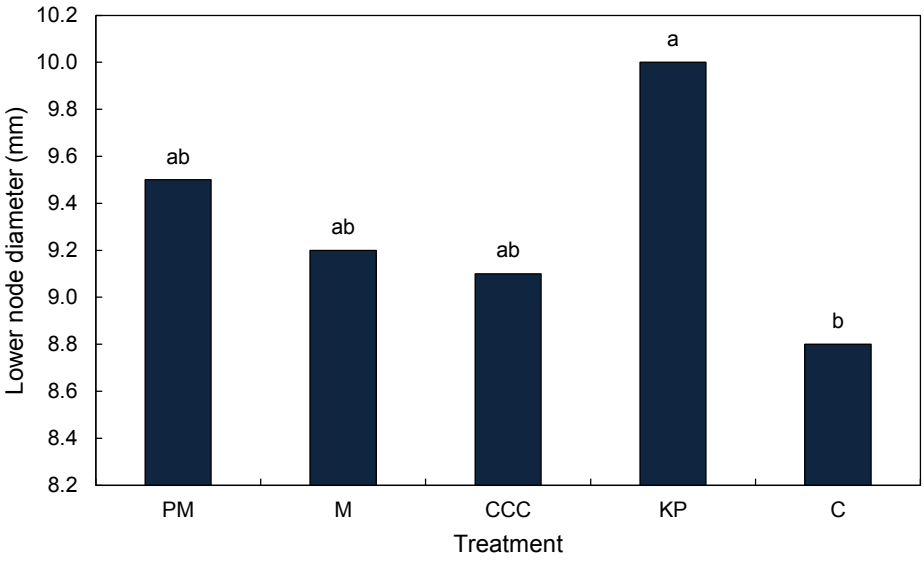

Figure 5.6: Lower node diameter (mm) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 110 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

Above ground dry mass

The mean above ground dry mass (leaves and stems) did not differ significantly between Langgewens and Altona (Table 5.2), but the above ground dry mass of Hyola 555TT plants were significantly affected by the application of PGRs (Figure 5.7). Compared to the control all four PGR treatments tend to increase the above ground dry mass at 110 days after planting, but only Kelpak®-treated plants were significantly heavier than the control. When compared to the control, Kelpak®-treated plants increased the above ground dry mass with almost 43%, while Primo MAXX®, Moddus® 250 EC and CeCeCe® 750 increased the above ground dry mass with approximately 11%, 10% and 16% respectively.

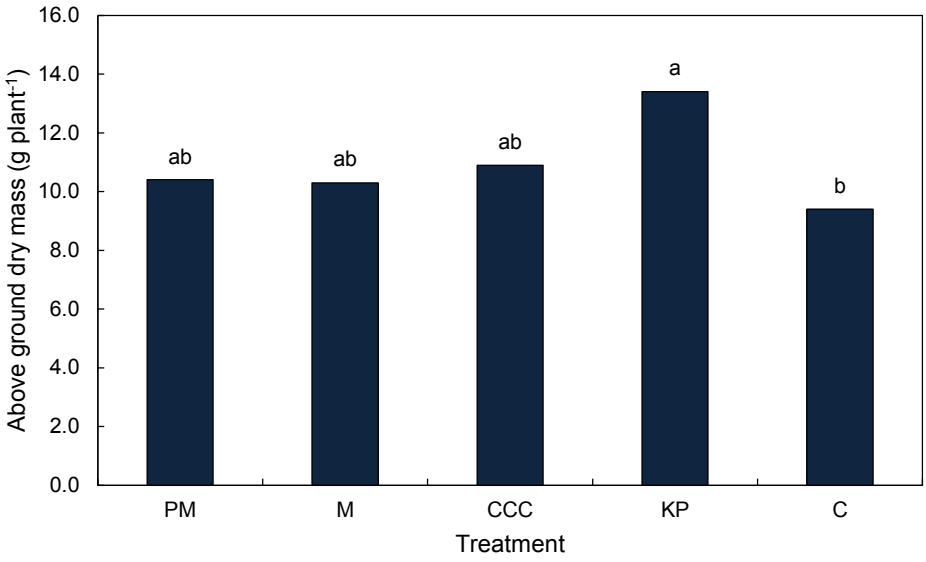

Figure 5.7: Above ground dry mass (g plant^{-1}) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 110 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

Root dry mass

At 110 days after planting the mean root dry mass were not affected by locality (Table 5.2) or the different PGR treatments (Figure 5.8). Control and Kelpak®-treated plants showed a root dry mass of 2.0 g plant^{-1}, while Moddus® 250 EC-treated plants produced a root dry mass of 1.5 g plant^{-1}.

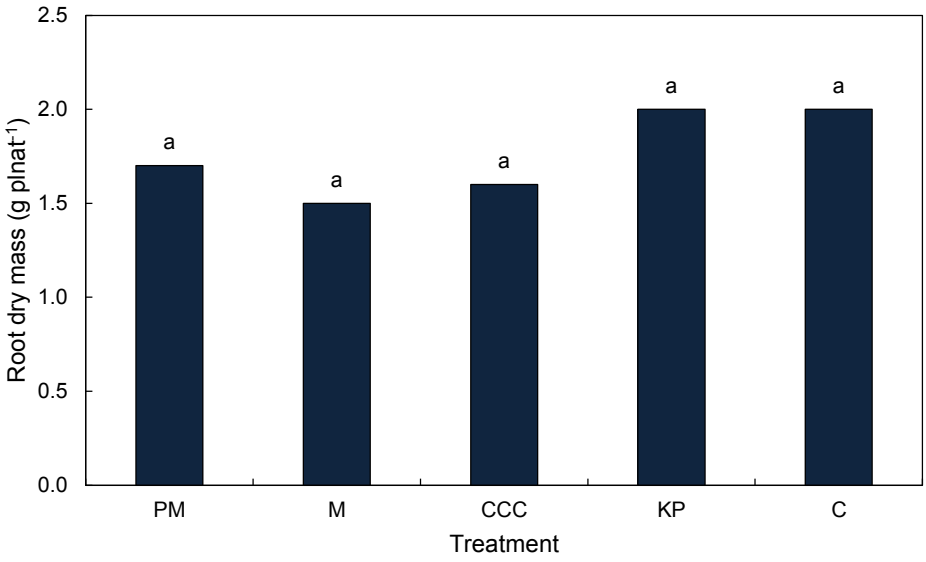

Figure 5.8: Root dry mass (g plant^{-1}) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 110 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

5.3.1.2. Effect of plant growth regulators on reproductive growth

Flower numbers

Number of flowers plant^{-1} at Langgewens (308.8 plant^{-1}) was significantly more than that at Altona (233.1 plant^{-1}) (Table 5.2). At 110 days after planting, the number of flowers plant^{-1} was not significantly affected by the different treatments, although all PGR treatments tend to produce a larger number of flowers plant^{-1} than the control. Kelpak®-treated plants showed the largest number of flowers plant^{-1} - almost 47% more than the control (Figure 5.9).

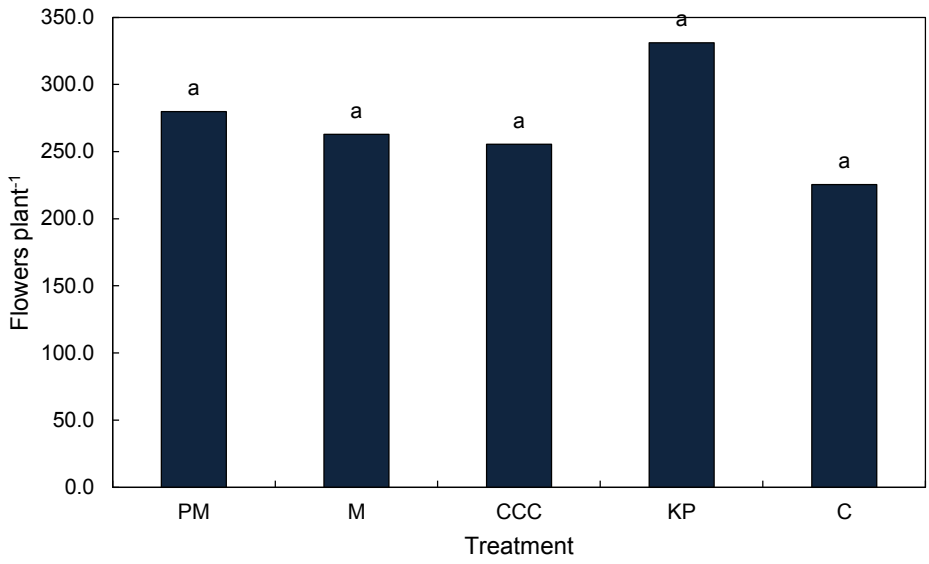

Figure 5.9: Number of flowers plant^{-1} of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 110 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

Pod numbers

Compared to Altona, Langgewens increased the number of pods plant^{-1} with almost 90% at 110 days after planting (Table 5.2), but PGR treatments did not have a significant effect (Figure 5.10).

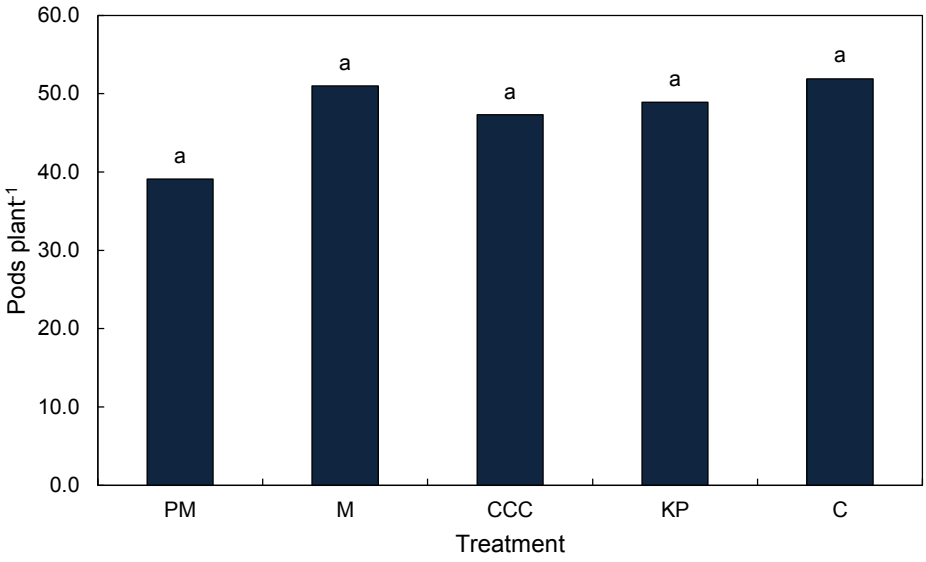

Figure 5.10: Number of pods plant^{-1} of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 110 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

5.3.2. Sampling at 125 days after planting

5.3.2.1. Effect of plant growth regulators on vegetative growth

Plant height

On the 125th day after planting (49 days after treatment), the mean plant height of canola cultivar Hyola 555TT did not differ significantly between Langgewens and Altona (Table 5.2), but PGR treatments had a significant effect (Figure 5.11). Kelpak®- and CeCeCe® 750-treated plants were significantly larger than plants treated with Primo MAXX®, but not significantly larger than the control or Moddus® 250 EC-treated plants. On average Primo MAXX® tends to decrease the plant height with 6% compared to the control.

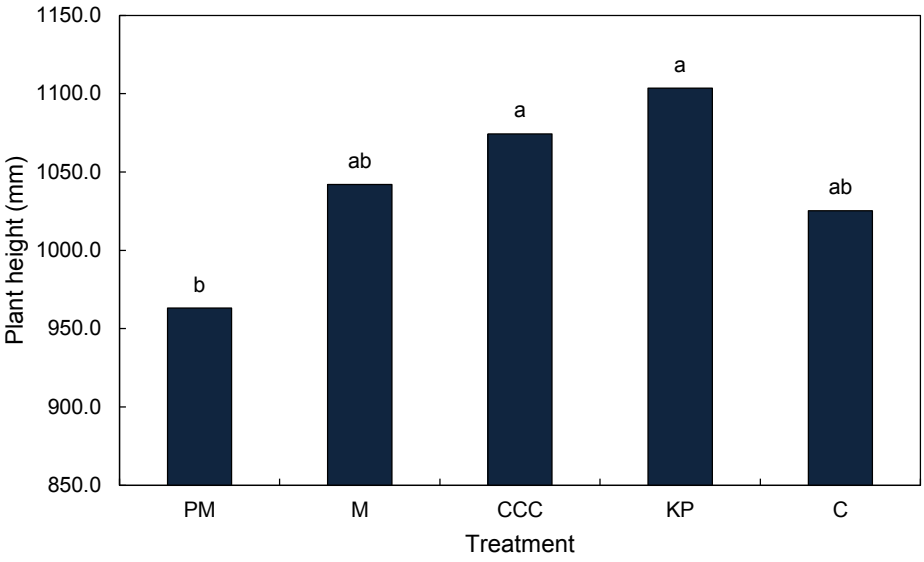

Figure 5.11: Plant heights (mm) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 125 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

Above ground dry mass

The mean above ground dry mass (leaves and stems) at 125 days after planting was not affected by locality (Table 5.2), but PGR treatments tend to increase the above ground dry mass when compared to the control (15.7 g plant^{-1}), with Primo MAXX®-treated plants (22.2 g plant^{-1}) differing significantly (Figure 5.12). When compared to the control PGRs, CeCeCe® 750, Kelpak®, Moddus® 250 EC and Primo MAXX® increased the above ground dry mass with approximately 22%, 23%, 32% and 41%, respectively.

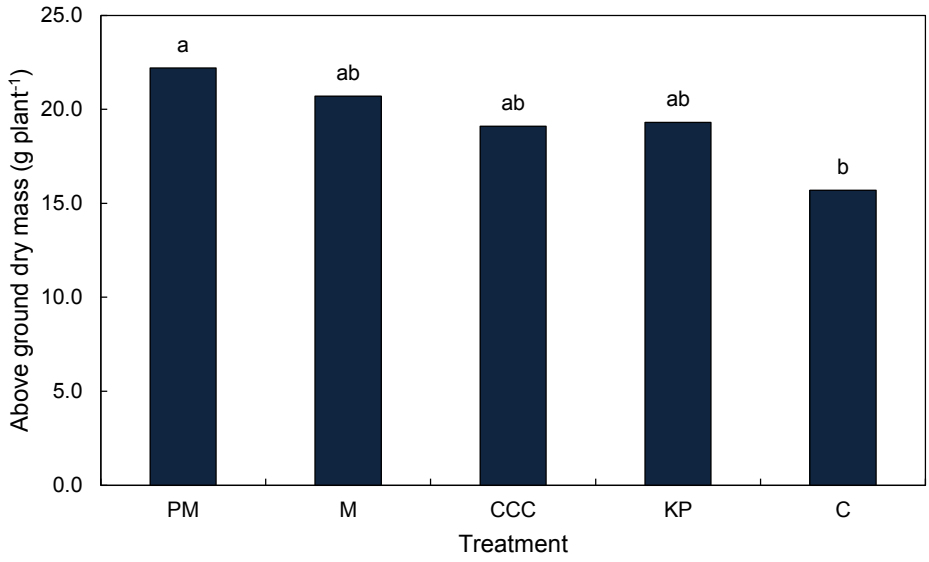

Figure 5.12: Above ground dry mass (g plant^{-1}) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 125 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

Root dry mass

The mean root dry mass did not differ significantly between Langgewens and Altona at 125 days after planting (Table 5.2), but from Figure 5.13, it became clear that the PGRs had a significant effect on the root dry mass. As in the case of the above ground dry mass, all PGRs resulted in larger root dry mass values than the control (1.2 g plant^{-1}), but only Primo MAXX®-treated plants (2.0 g plant^{-1}) were significantly different. When compared to the control PGRs Moddus® 250 EC, CeCeCe® 750 and Kelpak® increased the root dry mass with approximately 42%, 25% and 17% respectively, while Primo MAXX® increased root dry mass with approximately 67%.

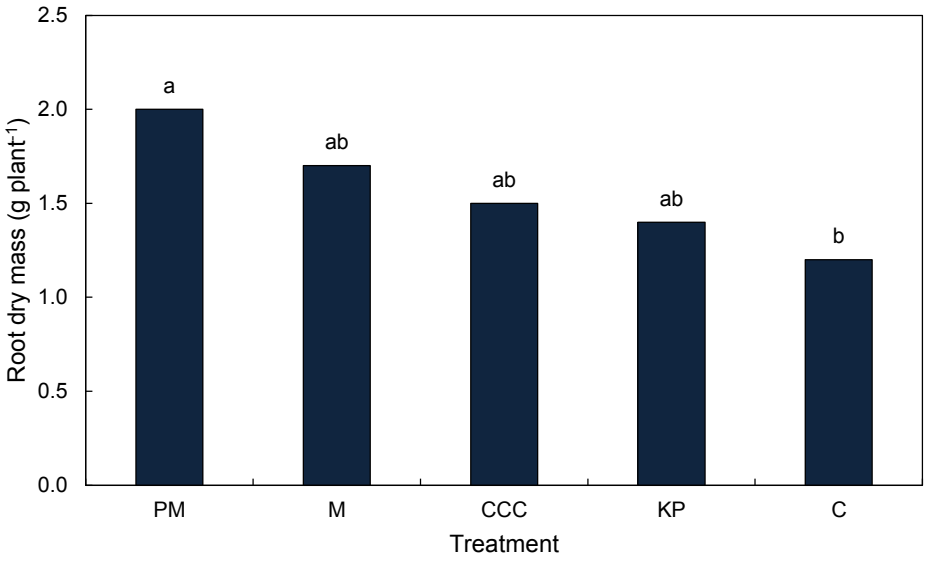

Figure 5.13: Root dry mass (g plant^{-1}) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 125 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

5.3.2.2. Effect of plant growth regulators on reproductive growth

Pod numbers

The number of pods plant^{-1} at 125 days after planting at Langgewens (212.9 pods plant^{-1}) was significantly more than that at Altona (139.8 pods plant^{-1}) (Table 5.2). All PGR treatments showed a significant larger number of pods plant^{-1} when compared to the control (Figure 5.14), but no significant differences were shown between different PGR treatments. Primo MAXX®, Kelpak®, CeCeCe® 750 and Moddus® 250 EC increased the number of pods plant^{-1} with approximately 50%, 50%, 49% and 40% respectively, when compared to the control.

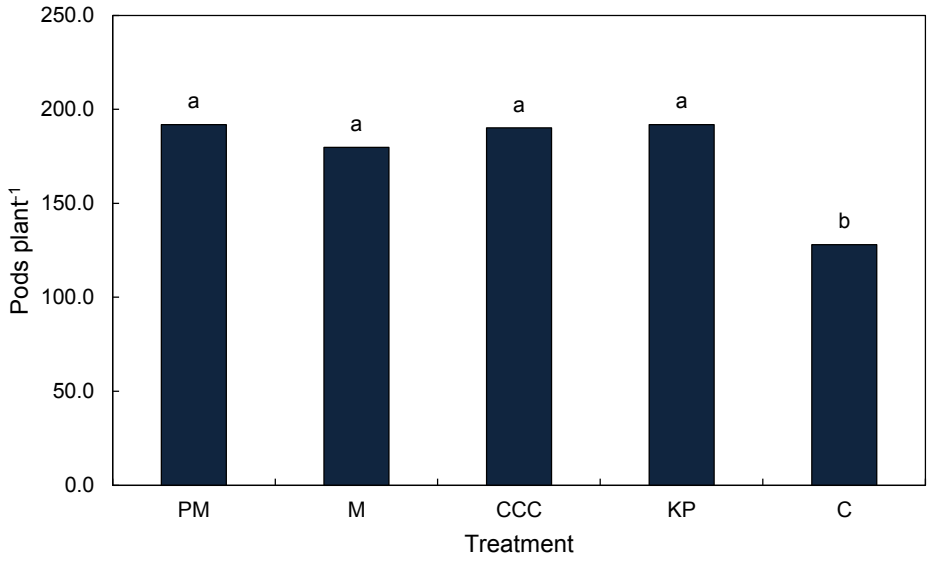

Figure 5.14: Number of pods plant^{-1} of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 125 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

5.3.3. Sampling at 195 days after planting

5.3.3.1. Effect of plant growth regulators on reproductive growth

Mass seed^{-1}

During the final harvesting at 195 days after planting (119 days after treatment), mass seed^{-1} (mg) at Roodebloem (3.2 mg) was significantly higher than that at Langgewens (2.7 mg) and Altona (2.5 mg) (Table 2.5). No significant differences were shown between the treatments (data not shown).

Grain yield (ton ha^{-1})

At Altona, Hyola 555TT produced a significantly larger grain yield (ton ha^{-1}) than at Langgewens and Roodebloem (Table 5.2). Although grain yield did not differ significantly due to the treatments applied (Figure 5.15), all PGR treatments tend to increase the yield when compared to the control. With trinexapac-ethyl as their active ingredient, Primo MAXX® and Moddus® 250 EC showed the largest yield of 1.764 ton ha^{-1} which was on average 4% higher than that of control plots (1.691 ton ha^{-1}).

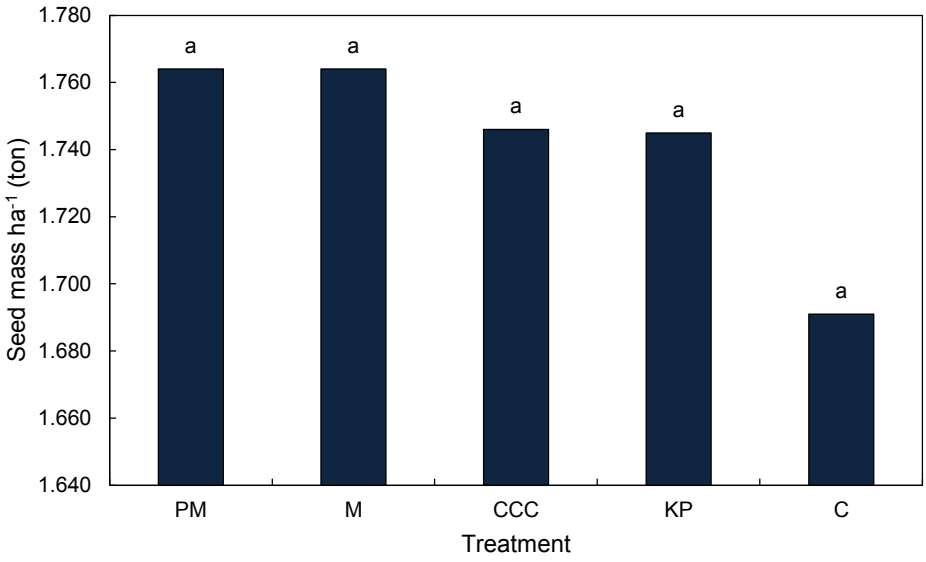

Figure 5.15: Grain yield (ton ha^{-1}) of canola cultivar Hyola 555TT as affected by plant growth regulator treatments at 195 days after planting

PM = Primo MAXX®, M = Moddus® 250 EC, CCC = CeCeCe® 750, KP = Kelpak®, C = Control. *Bars with the same letter are not significantly different at p=0.05 probability level*

5.4. Discussion

5.4.1. Kelpak®

The application of Kelpak® in field trials done on canola during 2013 in the Western Cape increased the lower node diameter and above ground dry mass significantly (110 days after planting). These results support those of Nelson and van Staden (1984), which showed that Kelpak® increases the culm diameter of wheat grown under growth chamber conditions. In general Kelpak® tends to enhance vegetative growth when compared to the control. Although not significant, Kelpak® tends to result in taller plants with larger leaf areas $plant^{-1}$. Khan et al. (2009) also reported an increase in leaf area and plant chlorophyll content using seaweeds and seaweed products. Bore and Ng´etich (2007) and Papenfus et al. (2013) reported similar results with Kelpak® on nutrient-stressed okra seedlings, tepary beans, and nursery tea seedlings.

In contrast to results with nursery tea seedlings (Bore and Ng´etich 2007), root dry mass of canola on average, did not increase significantly due to the application of Kelpak® in the field trials done at Langgewens and Altona during 2013, most

probably due to the extremely stony soil at these localities which made the measurement of roots very difficult.

In the field study done on canola, Kelpak® showed a tendency to delay the onset of flowering and pod development as it produce a higher number of flowers plant^{-1} and lower number of pods plant^{-1} at 110 days after planting, but significantly more pods plant^{-1} at 125 days after planting when compared to the control.

Kelpak® did not increase canola grain yields (ton ha^{-1}) significantly during the 2013 field trials done at Langgewens, Roodebloem and Altona when compared to the control, but studies done by Ferreira and Lourens (2002) indicated that foliar application with Kelpak® (2 L ha^{-1}, applied at 3- or 5-leaf stages) may result in a significant increase in canola yield. According to Papenfus et al. (2013) production cost and the usage of chemical fertilizers can potentially be reduced using Kelpak® as plant growth stimulator, but it is of utmost importance to use the correct concentration of seaweed extracts, because high concentrations may result in toxicity (Ferreira and Lourens 2002).

5.4.2. CeCeCe® 750

In contrast to studies done on chlormequat chloride alone or in PGR combinations (Armstrong and Nicol 1991; Giridhar and Giri 1997; Sanvicente et al. 1999; Gans et al. 2000; Rajala and Peltonen-Sainio 2001; Aamlid et al. 2007; Haque et al. 2007; Zhang et al. 2009; Spitzer et al. 2011; Gebre et al. 2012; Matysiak and Kaczmarek 2013), CeCeCe® 750 did not reduce height of canola plants during the 2013 field trials done at Langgewens and Altona, when compared to the control. In general CeCeCe® 750 did not have a large effect on vegetative growth when compared to the control in this study. The difference in lower node diameter between CeCeCe® 750 and control plants were also relatively small if any, supporting results on *Eragrostis tef* (Gebre et al. 2012). Previous studies done on winter barley however showed an increase in lodging resistance by means of thickening the culm wall after applying chlormequat chloride at the beginning of stem elongation (Sanvicente et al. 1999).

During the field trials at Langgewens and Altona (2013), the application of CeCeCe® 750 on canola tends to result in higher numbers of flowers plant^{-1} and lower numbers of pods plant^{-1} at 110 days after planting, but a significantly higher number of pods

plant^{-1} at 125 days after planting compared to the control. Grain yields also tend to be higher. These results supported earlier results which showed that chlormequat chloride tend to delay onset of flowering and pod development (Spitzer et al. 2011), but increases grain yield (Giridhar and Giri 1997). In addition, Giridhar and Giri (1997) reported an improvement in both protein and oil yields after spraying groundnut plants with "CCC" (chlormequat chloride applied at 0.5 mL L^{-1} water).

5.4.3. Primo MAXX® and Moddus® 250 EC

In the canola field trial done at Langgewens and Altona in 2013, Primo MAXX® tends to produce shorter plants when compared to control and other PGR treated plants. As also shown in a study where canola (*B. napus* L.) was treated with "Moddus" 222.0 g L^{-1} trinexapac applied at 0.5 L ha^{-1} (Ijaz and Honermeier 2012), the application of Moddus® 250 EC tends to reduce plant height at 110 days after planting, but not at 125 days after planting. In earlier studies (Matysiak 2006; Li et al. 2011; Wiersma et al. 2011; Ijaz and Honermeier 2012) where trinexapac-ethyl was applied alone or in combination with chlormequat (CCC), plant height were reduced, while a study done on spring wheat (Wiersma et al. 2011), showed that an increase in trinexapac-ethyl levels resulted in a linearly reduction in plant height, while stem strength and plant erectness were increased linearly.

In accordance to Matysiak (2006) and Wiersma et al. (2011), the application of Primo MAXX® and Moddus® 250 EC tend to increase the lower node diameters during 2013 field trials with canola. However, opposing earlier studies (Matysiak 2006), trinexapac-ethyl treatments significantly increased both above ground and root dry mass at 125 days after planting when compared to the control. Previous reports of trinexapac-ethyl effects on root growth varied from improved growth (Ijaz and Honermeier 2012) to no response (Rajala et al. 2002; McCann and Huang 2007).

The application of Primo MAXX® and Moddus® 250 EC tend to produce a higher number of flowers plant^{-1} and lower number of pods plant^{-1} at 110 days after planting and significantly more pods plant^{-1} at 125 days after planting when compared to the control. This higher number of pods plant^{-1} resulted in grain yield of 1.764 ton ha^{-1} with Primo MAXX® and Moddus® 250 EC compared to the 1.691 ton ha^{-1} from control plots, supporting the results obtained with winter wheat (Matysiak 2006). According to Espindula et al. (2009) and Li et al. (2011) grain yield increases may be

ascribed to morphological changes in the plants architecture, induced by trinexapac-ethyl as discussed.

5.5. Conclusion

Although plant growth and grain yield did differ between different localities in 2013, no significant locality × treatment interaction was recorded. Similar responses to PGR applications were thus found at all localities in spite of substantial differences in growing conditions.

No significant differences in plant height was recorded in field trials with canola during 2013, but Primo MAXX® tend to reduce plant height more efficiently when compared to Moddus® 250 EC, CeCeCe® 750 and Kelpak®. Primo MAXX® and Kelpak® had the largest effect on vegetative growth as Kelpak® resulted in a significant increase in the lower node diameter and above ground dry mass at 110 days after planting, while Primo MAXX® significantly increased the above ground and root dry mass 125 days after planting when compared to the control. All PGRs tested, increased the number of pods plant^{-1} significantly at 125 days after planting and tend to increase the final grain yield (ton ha^{-1}).

With the exception of Kelpak®, none of the PGRs tested in this study is at present registered for use on canola in South Africa. It is for this reason quite possible that dosage rates and application protocols used were not optimal for use on canola.

Due to promising results obtained in 2013 it is clear that more research needs to be done as PGRs response may be dependent on the cultivar, PGR combinations, application timing and rates. Furthermore, economic analysis needs to be conducted to determine the cost benefit ratio of PGR usage.

5.6. References

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Chapter 6
Summary and General Conclusions

Anti-lodging plant growth regulators (PGRs) are synthetic compounds, primarily used to reduce unwanted longitudinal shoot growth and lodging in agricultural crops, while either preserving or enhancing productivity. Previously PGRs have experimentally, successfully been used to reduce canola plant height and lodging in Australia. In spite of this, the commercial use of anti-lodging PGRs on canola cultivars is limited due to lack of scientific data. The objective of this study was to determine the effect of anti-lodging PGRs on the agronomic and quality characteristics of commercial canola cultivars (*Brassica napus* L.), under South African conditions.

The present study consists of two glasshouse trials presented in Chapter 3: "Influence of anti-lodging plant growth regulators on growth and yield of glasshouse grown canola (*Brassica napus* L.) in sandy soil" and Chapter 4: "Efficacy of anti-lodging plant growth regulators on growth and yield of glasshouse grown canola (*Brassica napus* L.) under optimum growth conditions" as well as a field trial presented in Chapter 5: "Effect of anti-lodging plant growth regulators on growth and yield of canola (*Brassica napus* L.) grown at different localities in the Western Cape Province of South Africa".

The glasshouse trials were conducted using 3.0 L pots under temperature-controlled conditions (15/10˚C day/night) at the Department of Agronomy at Stellenbosch University. Glasshouse trials comprised of a randomized complete block design with a factorial combination of two cultivars of spring canola (Hyola 555TT and 43C80) and four PGR treatments, applied at growth stage 3.1 (budding stage) using an automated cabinet sprayer. In the first trial, Kelpak® (in combination with wetting agent Foliwett® 900); CeCeCe® 750; Moddus® 250 EC and a control (untreated) treatment were used. During the second trial three changes were made: firstly PGR Moddus® 250 EC was replaced with Primo MAXX®; secondly all PGRs were applied in combination with Foliwett® 900; and thirdly the growing medium (coarse sand) was replaced with a 1:1 combination of coarse sand and compost for optimum growth conditions.

The field trial was conducted at Langgewens, Altona and Roodebloem Research Farms using one cultivar of spring canola (Hyola 555TT) and comprised of a

randomized complete block design with a factorial combination of five PGR treatments, replicated four times: Kelpak® (in combination with Foliwett® 900), CeCeCe® 750, Moddus® 250 EC, Primo MAXX® and control (untreated). Treatments were applied at growth stage 3.1 (budding stage) using a 20 L knapsack sprayer.

Kelpak®

The application of Kelpak® significantly increased the above ground dry mass of Hyola 555TT in the glasshouse trial done in sandy soil as well as during the field trial at respectively full flower (93 days after planting) and lower pod filling stage (110 days after planting), when compared to the control. However, in contrast the root dry mass remained unaffected under field conditions; possibly due to the stony soil which made the measurement of roots challenging. Moreover, contradictory results were shown at lower pod filling stage in the glasshouse trial, as above ground dry mass of Kelpak®-treated Hyola 555TT plants decreased significantly in the sandy soil. With the exception of lower node diameter during lower pod filling stage (110 days after planting) and number of pods plant^{-1} during full pod filling stage (125 days after planting) that were significantly increased under field conditions and a tendency to improve vegetative growth, Kelpak® treatments did not have any significant effect on growth and yield of canola. Since Kelpak® did not reduce plant height in this study it can be concluded that it will most probably not reduce lodging in canola.

CeCeCe® 750

Though showing significant contrasting effects between cultivar Hyola 555TT and 43C80 at full flower (93 days after planting) in sandy soil in the glasshouse, CeCeCe® 750, generally did not reduce plant height. Whilst the leaf area and above ground dry mass plant^{-1} of Hyola 555TT was significantly increased in the sandy soil at flowering (93 days after planting), CeCeCe® 750 significantly decreased the overall above ground dry mass during pod filling (114 days after planting), compared to the control. Under field conditions the effect of CeCeCe® 750 treatments showed only during pod filling (125 days after planting) when the number of pods plant^{-1} were significantly increased, compared to the control. Although grain yields tend to increase in the field trial, CeCeCe® 750 applications generally did not have a large effect on growth, yield or plant height of canola and for this reason do not show much potential to decrease lodging in canola.

Moddus® 250 EC

Although Moddus® 250 EC tend to reduce plant height and increase the lower node diameter during the glasshouse trial in sandy soil and the field trial, Moddus® 250 EC in general, failed to have a significant effect on plant height and therefore resistance to lodging. During the glasshouse trial in sandy soil Moddus® 250 EC resulted in a significant reduction in above ground dry mass of cultivar 43C80 at 114 days after planting (pod filling stage). Under field conditions an increase in number of pods plant^{-1} was however recorded which resulted in a tendency to increase grain yield. Presumably, this might be the accumulated result of various insignificant morphological changes in the plants architecture induced by Moddus® 250 EC.

Primo MAXX®

Primo MAXX® showed the most promising results with regard to the decrease in plant height in both the glasshouse trial under optimum conditions and the trial conducted under field conditions in the canola production area of the Western Cape. The decrease in plant height is most probably due to the reduced rate of cell elongation and division initiated by Primo MAXX®. In addition to that Primo MAXX® also increased the lower node diameter of 43C80 and number of flower stalks plant^{-1} in the glasshouse trial under optimum conditions. This increase in lower node diameter may reduce lodging, while the increase in number of flowers may suggest a higher yield potential. Unfortunately, Primo MAXX® reduced the pod dry mass plant^{-1} and mass pod^{-1} of 43C80 in the same trial. However, it should be taken in consideration that no final grain yield was measured in this glasshouse trial. Reduced pod dry mass and mass pod^{-1} measured at 114 days after planting when only seeds in the lower pods became green-brown mottled (growth stage 5.3) may therefore be the result of a delay in the rate of pod filling and retarded ripening due to the Primo MAXX® application and not a true indication of grain yield potential.

Although the reduction of plant height of Primo MAXX® treated plants also showed a reduced above ground dry mass in the glasshouse trial under optimum conditions, this was not true for the trial under field conditions. The field trials conducted at three localities in the canola production area of the Western Cape showed a significant increase in above ground dry mass, root dry mass and number of pods plant^{-1} at 125

days after planting and a tendency to increase the grain yield at 195 days after planting.

The improved response achieved by Primo MAXX® (trinexapac-ethyl 120 g L^{-1}) application compared to Moddus® (trinexapac-ethyl 250 g L^{-1}) may be ascribed to the higher application rate used (Primo MAXX® applied at 10 × the application rate of Moddus®).

Conclusions and future research

This study showed that Primo MAXX® without any doubt has the potential to reduce plant height in canola which may suggest better resistance to lodging. Because Primo MAXX® treated plants also produce more flowers and pods, grain yields may also increase. However, since only PGR Kelpak® is at present registered for use on canola in South Africa, it is quite possible that dosage rates and application protocols used for CeCeCe® 750, Moddus® 250 EC and Primo MAXX® might not have been optimal for use in canola. For this reason it is recommended that further research be done to establish the optimum timing and rates of PGRs for canola. Additionally, research is required to determine whether the response is cultivar specific and whether different PGRs can be combined. Finally, an economic analysis needs to be done to determine the cost benefit ratio of PGR usage.